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Cornell University Medical College

Studies

from the

Departments

of

Pathology Bacteriology

Experimental Pathology

Experimental Therapeutics

VOLUME XIV

1914

NEW YORK CITY

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No. 1

STUDIES IN ANAPHYLAXIS.*

RICHARD WEIL

In the present series of studies a number of independent problems are separately considered. These problems, however, are intimately related to one another, and the solution of each of them is essential to the understanding of certain aspects of immunity.

Theoretical discussion has been added, in which certain of the phenomena of immunity are analyzed upon the basis of the experimental results herein described.

VII. THE RELATION BETWEEN ANTIBODY CONTENT AND LETHAL DOSE IN ANAPHYLAXIS.

In the present study the attempt has been made to determine whether there is any quantitative relationship between the amount of antibody which a sensitized animal possesses and the amount of antigen which will be required to produce acute death in that animal. This problem has been approached by means of two methods, namely, first, the intravenous injection of the living animal, and, second, the study of the isolated uterine muscle preparation. All of the animals were sensitized by the passive method, inasmuch as this procedure alone permits of accurate control of the amount of antibodies in the body of the guinea-pig. Actively sensitized guinea-pigs are not suitable for this investigation, inasmuch as they differ very materially in the amount of antibody production, even though the same initial amount of antigen is introduced to sensitized them.

As regards the relative amounts of antigen required to produce death in guinea-pigs passively sensitized by varying amounts of the same immune serum there are no experimental data in the literature. Doerr and Russ, indeed, asserted that there was

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an inverse ratio between these quantities, but made no direct experiments to demonstrate this theory. In the following tables are presented the determinations of the minimal fatal, or anaphylactic, dose of horse serum, in two series of guinea-pigs passively sensitized with different amounts of the serum of a rabbit which had been immunized against horse serum. The amount of rabbit's immune serum given in the first series of guinea-pigs was two-tenths cubic centimeter; the amount given in the second series was two cubic centimeters, or ten times the quantity given in the first series. Both series received the serum intraperitoneally and at the same time. The weights of all the guinea-pigs were approximately the same, varying from one hundred and fifty to two hundred and ten grams. The determination of the minimal anaphylactic dose of horse serum was undertaken in both series on the first day after sensitization. The minimal sensitizing dose of the rabbit serum employed had been previously found to be one-tenth cubic centimeter, so that the first series received two M.S.D. (minimal sensitizing doses), the second series twenty M.S.D.

TABLE I.

The guinea-pigs had received an intraperitoneal injection of .2 cubic centimeter of Rabbit Serum 747 intraperitoneally.

G.P.	Weight in Grams.	Dose of Antigen Intravenously.	Result.
1	150	0.06 cc.	Moderate symptoms.
2	185	0.08 "	Mild symptoms.
3	173	0.09 "	Severe symptoms.
4	165	0.1 "	†
5	190	0.2 "	†

One-tenth cubic centimeter was the minimal sensitizing dose (M.S.D.) of this serum. The animals were all tested on the first day after sensitization.

TABLE II.

The animals of this series were sensitized by 2 cubic centimeters of S. 747.

G.P.	Weight in Grams.	Dose of Antigen Intravenously.	Result.
1	150	0.01 cc.	No symptoms.
2	204	0.02 "	Delayed symptoms.
3	211	0.04 "	†
4	183	0.06 "	†
5	206	0.08 "	†
6	180	0.1 "	†

Animals all tested on the first day after sensitization.

A comparison of the results yielded by these two experiments reveals the fact that the animals sensitized by means of smaller amounts of immune serum require a larger amount of antigen to produce death than do the animals which had received a considerably larger amount of the same immune serum. Whether definite quantitative ratios can be established to cover the relationship between these two factors has not been determined. At all events, it is clear from the present series that an inverse relationship exists.

In the second series of animals the same experiment was performed, using a different rabbit's serum, the M.S.D. of which was .15 cubic centimeter. In the present series the intravenous injection was made on the third day after sensitization. The results obtained in this series are presented in Tables III. and IV.

TABLE III.

The animals of this series received an intraperitoneal injection of .2 cubic centimeters of Rabbit Serum 155 intraperitoneally.

G.P.	Weight in Grams.	Dose of Antigen Intravenously.	Result.
1	190	0.005 cc.	Mild symptoms.
2	179	0.01 "	" "
3	205	0.02 "	Moderate symptoms.
4	160	0.03 "	†
5	175	0.05 "	†

TABLE IV.

The animals of this series received two (2) cubic centimeters of Serum 155 intraperitoneally.

G.P.	Weight in Grams.	Dose of Antigen Intravenously.	Result.
1	177	0.005 cc.	Mild symptoms.
2	172	0.01 "	Convulsions, recovered.
3	175	0.01 "	†
4	231	0.02 "	†

These pigs were tested on the third day after sensitization.

The data of these four sets of experiments are summarized in Table V.

TABLE V.

Sensitizing Dose of Immune Serum.	Minimal Fatal Dose of Antigen.
0.2 cc. S. 747.	0.1 cc. horse serum.
2.0 " S. 747.	0.04 " " "
0.2 " S. 155.	0.03 " " "
2.0 " S. 155.	0.01 " " "

Animals tested on the third day after sensitization.

The conclusion is obvious that in these four groups of animals the greater the sensitizing dose the smaller is the minimal fatal dose of antigen.

The attempt has been made to determine the same data for the isolated uteri of sensitized animals. Except for the fact that the antigen is tested against the muscle preparation instead of against the living animal, the method is essentially similar to that previously described. A series of guinea-pigs are passively sensitized by the intraperitoneal injection of graded amounts of the serum of a rabbit immunized against horse serum. After the lapse of a sufficient interval to permit of absorption of the alien serum, the guinea-pigs are killed and in each case the minimal dose of antigen required to produce a distinct uterine contraction is determined. This quantity is briefly described as the minimal anaphylactic dose by Dale's method, or, abbreviated, as M.A.D.(D.). This determination does not, of course, correspond accurately to that determined in vivo as the minimal fatal dose, for the reason that the data yielded by the two methods are not identical. The use of the living animal, when death is accepted as the criterion, determines that amount of antigen which is required to produce a cellular discharge of sufficient violence to kill the animal. The muscular preparation, however, determines the amount of antigen required to produce the least distinct muscular contraction. The latter method, therefore, is considerably more delicate. And yet, as will subsequently be shown, the conclusions to be drawn from the use of the two methods are identically the same.

In the use of Dale's method for quantitative reactions certain precautions must be kept in mind. It is essential in the first place that the uteri should be examined under similar conditions. The load attached, the length of the multiplying lever, and the amount of fluid in which the preparation is suspended should, of course, be the same. The organs should be removed from the animal immediately after death and the antigen tested within ten to fifteen minutes. If the uterus is kept for a half-hour or longer in aerated Locke's solution before being tested it will frequently be found that its sensitiveness to the antigen is increased. In the series herein reported one uterus was always examined immediately after death, while the other was kept in

Locke's fluid for a second series of determinations. When this is done the second set of tests of the entire series must be compared with each other and not with the results yielded by the first uterine preparation. Furthermore, it is always necessary to test at least three guinea-pigs with each amount of immune rabbit's serum in order to exclude individual variations. In testing out each uterus it is advisable to proceed from the smaller doses of antigen up to the larger, in order not to desensitize the muscle by means of a large dose before the smaller dose has been tested. Partial desensitization of the muscle, such as is produced by larger doses, distinctly raises the minimal anaphylactic dose. Furthermore, as may be seen in many of the tracings, there is a curious mode of action of the muscle preparation which must always be reckoned in quantitative work. The muscle is not in the most favorable condition for responding to the minimal anaphylactic dose until after it has already responded to a somewhat larger dose. Thus, the preparation may at first refuse to contract upon the addition of one-thousandth cubic centimeter of horse serum, whereas it gives a good response to that amount after it has previously been stimulated by one-hundredth cubic centimeter. Therefore, it is always necessary to retrace one's steps and make a second trial of smaller quantities than have been determined upon the first trial. It is, of course, always advisable to wash out the apparatus twice with Locke's fluid after the addition of anything more than minimal test doses of the antigen. Moreover, if the most accurate results are to be obtained the uterus should, as a rule, not be permitted to register the full length of a powerful contraction, but as soon as the desired effect is evident the fluid should be removed and the preparation thoroughly washed. In this way it becomes possible to minimize the disturbing factor of desensitization. The modifying influence of certain of these factors is illustrated in some of the following tracings.

The experiment which is illustrated by the curves accompanying this paper was carried out on the following plan: Two rabbits which had been highly immunized against horse serum by means of repeated intravenous injections were freely bled on the same day. These two sera were mixed and the minimal sensitizing dose (M.S.D.) for guinea-pigs was determined. This dose, as has been described in previous papers, is the amount necessary

to prepare the guinea-pigs in such a way that they are killed by the intravenous administration of horse serum on the day succeeding sensitization. Sensitization is always performed by the peritoneal route. In this experiment it was found that two-tenths (.2) of a cubic centimeter was the minimal sensitizing amount. This having been determined, a series of female guinea-pigs were sensitized in immediate succession. The quantities of rabbits's serum given were as follows: one-tenth, three-tenths, one, two, and four cubic centimeters (.1, .3, 1, 2, 4 cubic centimeters.) Three pigs were sensitized with each of these amounts. Within five days after sensitization every uterus in the series was tested. The following curves represent the results:

The first three figures present curves obtained from pigs sensitized by one-tenth of a cubic centimeter of the immune serum. This is one-half of the M.S.D. The three figures are introduced in order fully to illustrate the mode of experimentation. It will be seen that in this series the addition of one-tenth cubic centimeter of horse serum regularly produces a distinct response. In one of the animals one-hundredth produces a sharp contraction, while in another the reaction to this amount is just perceptible. Therefore, the M.A.D.(D.) in this series of three figures lies between 1/10 and 1/100 cubic centimeter of the antigen.

FIGURE 1.—G.P. sensitized by .1 cubic centimeter serum of rabbit immunized to horse serum (R. vs.* H.S.). Uterus traced on fourth day. One-tenth cubic centimeter of horse serum produces a good contraction.

FIGURE 2.—G.P. sensitized by .1 cubic centimeter serum R. vs. H.S. Tested on fifth day. One-hundredth cubic centimeter of horse serum produces a sharp rise.

FIGURE 3.—G.P. sensitized by .1 cubic centimeter serum R. vs. H.S. Uterus, heavily overweighted, tested on fourth day. Slow response to .1 cubic centimeter of horse serum.

FIGURE 4.—G.P. sensitized by .3 cubic centimeter serum R. vs. H.S. Tested on second day. Reaction to one one-thousandth cubic centimeter of horse serum.

The series which received three-tenths of a cubic centimeter of immune rabbit's serum can be illustrated by a single curve, which demonstrates that one-thousandth of a cubic centimeter

*vs.=versus.

of horse serum produces a distinct response. This was the average figure found for the uteri of this series.

The uterine tests upon guinea-pigs sensitized by one cubic centimeter of immune rabbit's serum are illustrated by means of Figures 5 and 6. The first of these curves is introduced for the purpose of illustrating the use of an inverted mode of procedure intesting the antigen, the larger doses being applied first. Under these conditions the uterine response becomes irregular and uncontrollable, and the dosage could not reliably be carried below 1/10,000 cubic centimeter of the antigen. The second figure shows that 1/1,000,000 stimulates a good uterine contraction.

FIGURE 5.—G.P. sensitized by 1 cubic centimeter serum R. vs. H.S. Tested on second day. Reaction to one ten-thousandth cubic centimeter of horse serum. The second reaction to one ten-thousandth cubic centimeter was very much more striking, but it is not reproduced on account of the length of the tracing.

FIGURE 6.—G.P. sensitized by 1 cubic centimeter serum R. vs. H.S. Tested on third day. Reaction to one-millionth of horse serum.

Figure 7, which is typical of its own series, shows that guinea-pigs sensitized by two cubic centimeters of immune rabbit's serum responds well to the application of 1/10,000,000 cubic centimeter of antigen.

FIGURE 7.—G.P. sensitized by 2 cubic centimeters serum R. vs. H.S. Tested on fourth day. Reaction to one ten-millionth of horse serum.

The series of pigs which received four cubic centimeters of immune serum is illustrated by Figures 8, 9, and 10. The first two illustrate the mode of reaction to amounts ranging from 1/100,000 to 1/10,000,000 cubic centimeter of antigen. The latent period with these small quantities is seen to be prolonged, as a rule, from two to three minutes. Undoubtedly this period is partly occupied by the diffusion of the serum through the one hundred and fifty cubic centimeters of fluid in which the uterus is suspended. An appreciable time must be occupied by this process, inasmuch as the serum is dropped into the reservoir at a distance of from one to two inches from the muscle. When a large amount, like 1/100 cubic centimeter is used, the response is very much more rapid.

The third curve was obtained with a heavily over-loaded uterine preparation in order to exclude the possible disturbing factor of spontaneous contractions. As a result the variations of level are small, and the latent period is prolonged to three minutes. The maximal contraction, produced by the addition of 1/100 cubic centimeter of horse serum, was correspondingly low and gradual.

FIGURE 8.—G.P. sensitized by 4 cubic centimeters serum R. vs. H. S. Tested on second day. Reaction to one-millionth.

FIGURE 9.—G.P. sensitized by 4 cubic centimeters serum R. vs. H. S. Traced on third day. Reaction to one-millionth; probably to one ten-millionth.

FIGURE 10.—G.P. sensitized by 4 cubic centimeters serum R. vs. H.S. Uterus, very heavily weighted, tested on third day. Reaction to one ten-millionth.

The results obtained from the study of these five series of guinea-pigs have been summarized in the following table:

Sensitizing Dose.	0.1	0.3	1.0	2.0	4.0
Minimal anaphylactic dose by Dale in cubic centimeters of horse serum...	1/100	1/1,000	1/1,000,000	1/10,000,000	1/10,000,000

Although it is not safe to make rigid quantitative deductions from the data here adduced it seems justifiable to draw certain general conclusions. In the first place it is clear that the uteri of animals sensitized by large amounts of immune rabbit's serum respond to very much smaller doses of the antigen than are required in the case of the uteri from animals which have received only small amounts of the serum. In other words, the minimal anaphylactic dose by Dale's method (M.A.D.(D.)) is, roughly speaking, in inverse ratio to the amount of immune body possessed by the animal. In this respect the result obtained by the graphic method corresponds exactly to that previously ascertained by means of intravenous injections into the living animal.

In the second place, it is clear that very large amounts of immune serum induce a degree of sensitiveness in the uterine

muscle which appears to be maximal; that is to say, it is not appreciably increased by the use of still larger amounts. Thus the uteri of animals which have been sensitized either with one, two or four cubic centimeters of serum alike react to amounts of horse serum ranging from one-millionth to one ten-millionth of a cubic centimeter.

It is, furthermore, evident that the uterus may be partially sensitized by a dose of immune serum which is insufficient to prepare the guinea-pig for a fatal anaphylactic response in vivo.

The amounts of antigen which suffice to produce a reaction in the muscle are astonishingly small, as was observed by Dale. If one considers that in each instance these amounts, as recorded, were actually diluted by one hundred and fifty (150) cubic centimeters of fluid, the delicacy of the reaction becomes almost inconceivable. Thus, the addition of one-millionth of horse serum represents a dilution of the antigen of one in one hundred and fifty millions (150,000,000), while a contraction may be elicited by even one-tenth of that concentration. It is safe to say that no other method permits of the detection of such infinitesimal amounts of the antigenic protein. Against this extreme delicacy of reaction to the specific antigen is to be set the fact that very considerable amounts of non-antigenic protein, even though biologically closely related, ordinarily produce no response. Thus, the muscle sensitized to horse serum fails to respond upon the addition of most other sera in amounts ranging from one-tenth to one-half cubic centimeter. There are, indeed, instances of crossed sensitization which will be described in a subsequent communication.

Conclusions. — 1. When guinea-pigs are passively sensitized by means of different amounts of heterologous (rabbit's) immune serum, the minimal fatal dose (M.A.D.) of antigen (horse serum) given intravenously is in an inverse relation to the amount of the previous sensitizing dose.

2. Whe the uteri of guinea-pigs prepared as above described are compared by Dale's method, it is found that the larger the sensitizing dose of immune serum the smaller is the amount of antigen required to produce a muscular contraction (M.A.D.(D.)).

3. The conclusion is drawn that the minimal anaphylactic dose is in an inverse ratio to the degree of sensitization of the guinea-pig.

4. Generally speaking, therefore, and in the absence of other disturbing factors, the relative amount of the minimal anaphylactic dose permits of an inference as to the amount of antibody possessed by a guinea-pig.

VIII. The function of circulating antibody and the avidity of cellular antibody.

In a previous paper it was shown that sensitized guinea-pigs could be partially protected against the anaphylactic effect of an intravenous injection of antigen, if the animals were first fortified by means of a large dose of immune serum. The quantitative relations, however, showed that circulating immune body protected only if present in enormously greater amount than the antibody already anchored by the cells of the body. Thus it was found that as much as thirty sensitizing doses of an immune rabbit's serum afforded protection against perhaps five or ten minimal fatal doses of antigen, but not against more. From this observation the conclusion was drawn that it was one of the normal functions of circulating antibody to protect the cells of the body from anaphylactic shock, but that this function was largely obscured, in case of the intravenous injection of antigen, by virtue of the heightened avidity of the cellular receptors. Subsequent experiments of the same type have fully confirmed the previous findings. In the present study an attempt has been made to penetrate somewhat further into the mutual relationships between the circulating and the anchored antibodies as regards their reaction to introduced antigen. Historically, it is of interest that both Pfeiffer and Sachs long ago maintained on theoretical grounds the doctrine of the heightened avidity of cellular receptors.

A series of guinea-pigs, ranging in weight from two hundred and thirty to two hundred and sixty-five grams, were passively sensitized by means of the intraperitoneal injection of one-tenth cubic centimeter of the serum of a rabbit immunized against horse serum. The fatal dose of horse serum for these animals, as determined by intravenous injections on the third day after sensitization, was .0075 cubic centimeter. On the same day four animals of the series were made the subject of a further experiment.

By way of preliminary to the following data, however, it is necessary to state that three guinea-pigs of the series were given intravenous injections respectively of .01, .03, and .05 cubic centimeter of horse serum. Immediately after death the uteri were removed and tracings were taken. It was found that the uterine preparation of the first of these animals responded moderately actively to horse serum, the second scarcely at all, and the third failed to contract upon the addition of .2 cubic centimeter of horse serum. These figures give the range for cellular desensitization by the intravenous route in controls.

Guinea-pig 1 was given an intravenous injection of two cubic centimeters of the immune rabbit serum intravenously. Immediately thereafter it received .01 cubic centimeter of horse serum intravenously. The animal manifested only very mild anaphylactic symptoms, and after the lapse of fifteen minutes it was exsanguinated. The centrifugalized serum was injected intraperitoneally into normal Guinea-pig 1a. The uterus of Guinea-pig 1 was at once removed and traced. It gave a sharp response to horse serum (Fig. 11). Guinea-pig 1a received an intravenous injection of .3 cubic centimeter of horse serum on the third day after its intraperitoneal injection and died in anaphylactic convulsions.

This experiment shows that twenty sensitizing doses in circulation sufficed to protect a guinea-pig against 1 1/4 M.A.D., that the uterus was not desensitized by this amount of antigen, and that sufficient antibody remained in circulation to sensitize a normal guinea-pig.

Guinea-pig 2 received three cubic centimeters of immune rabbit's serum intravenously, followed by one-tenth cubic centimeter of horse serum. The animal died at once in shock. The serum removed after death was injected intraperitoneally into Guinea-pig 2a. The uterine tracing shown in Figure 2 demonstrates that the cells were not completely desensitized. Guinea-pig 2a was given an intravenous injection of .5 cubic centimeter of horse serum three days later, and manifested severe symptoms. Owing to technical difficulties this animal had received only 1½ cubic centimeters of serum from Guinea-pig 2.

Guinea-pig 3 received two and one-half cubic centimeters of immune rabbit's serum intravenously, followed by .2 cubic centimeters of horse serum. The animal immediately died in con-

vulsions. Three cubic centimeters of serum obtained after death were injected into Guinea-pig 3a, and this animal was killed three days later by an intravenous injection of horse serum. The uterine tracings taken from both horns of Guinea-pig 3 are reproduced in Figures 13 and 14. They show practically complete desensitization of the cells.

Guinea-pig 4 received two and one-half cubic centimeters of immune rabbit's serum, followed by .4 cubic centimeter of horse serum. Death was immediate. The serum removed after death failed to sensitize Guinea-pig 4a completely, although this animal showed moderate symptoms on the injection of horse serum. The uterine tracings demonstrated complete desensitization, and do not require reproduction.

FIGURE 11.—G.P. sensitized by .1 cubic centimeter of Serum 435 (rabbit versus horse serum). On third day received intravenously 2 cubic centimeters of S. 435, followed by .01 cubic centimeter of horse serum. Killed after fifteen minutes.

FIGURE 12.—G.P. sensitized by .1 cubic centimeter S. 435. On third day 3 cubic centimeters S. 435, followed by .05 cubic centimeter of horse serum. Anaphylactic death.

FIGURE 13.—G.P. sensitized by .1 cubic centimeter S. 435. On third day 2.5 cubic centimeters S. 435, followed by .2 cubic centimeter of horse serum. Anaphylactic death.

FIGURE 14.—Same as Figure 13, opposite cornu.

The data obtained by these experiments are summarized in the following table:

TABLE 1.

G.P. 1..	2.0 cc. I.R.S. = 20 s.d. 0.01 " H.S. = 1.5 a.d.	Slight symptoms.	S + (G.P. 1a). C + (Fig. 11).
G.P. 2..	3.0 " I.R.S. = 30 s.d. 0.05 " H.S. = 6.5 a.d.	†	S + (G.P. 2a). C + (Fig. 12).
G.P. 3..	2.5 " I.R.S. = 25 s.d. 0.2 " H.S. = 26 a.d.	†	S + (G.P. 3a). C — (Figs. 13 and 14).
G.P. 4..	2.5 " I.R.S. = 25 s.d. 0.4 " H.S. = 52 a.d.	†	S + (G.P. 4a). C —

The following abbreviations are used in the above table: I.R.S., immune rabbit's serum; H.S., horse serum; s.d., sensitizing doses; a.d., anaphylactic or fatal doses; S, antibody content of serum; C, antibody content of uterine cells.

All of the above series had been sensitized by .1 cubic centimeter of immune rabbit's serum.

These results demonstrate again the relative inefficacy of the circulating antibody in protecting the sensitized guinea-pig against the anaphylactic effect of antigen when given intravenously. The mechanism of this inefficacy is also evident. The fact that in Guinea-pigs 3 and 4 the cells are completely desensitized, whereas the serum still contains a sufficient excess of free antibody to sensitize a normal guinea-pig passively, can only mean that the cellular antibody anchors antigen with much more avidity than does the circulating antibody. This conclusion is rendered even more striking by virtue of the fact that the cells contained only one twenty-fifth of the amount of antibody present in the circulating blood. On the other hand, it is clear that the circulating antibodies have at least in slight degree protected those of the cell, since complete desensitization was produced in the controls by .03 to .05 cubic centimeter of horse serum, whereas the same degree of uterine desensitization required much larger doses of antigen in the protected series.

These conclusions permit of the interpretation of two more or less obscure facts in anaphylaxis. In the first place, it is at once clear why an immunized guinea-pig should be anaphylactic when tested by intravenous injection of antigen. It is very difficult to immunize a guinea-pig against horse serum to such a degree that the circulating blood contains as many as twenty or thirty sensitizing units of antibody. Yet, as has been shown, this amount of antibody does not effectively protect against anaphylactic shock, when even moderate doses of antigen are injected.

In the second place the observation has often been made, and I have frequently had opportunity to confirm it, that an actively sensitized guinea-pig which has been desensitized or rendered anti-anaphylactic by means of the injection of a sublethal dose of antigen may still contain in its blood sufficient antibody to sensitize a normal guinea-pig passively. The preceding experiments offer a complete analogy to this observation. The sensitized guinea-pig contains free antibodies in the blood and fixed antibodies in the cells. The introduction of a minute amount of antigen serves to desensitize the latter, but not to saturate the former, by virtue of the demonstrated differences in avidity.

In a previous communication it was shown that an immunized guinea-pig, although typically hypersensitive to the intravenous administration of antigen, was nevertheless in most cases immune

to the intraperitoneal injection. From this observation it seemed reasonable to conclude that the circulating immune bodies protect the cells against antigen when the latter is administered by any except the intravenous route. The explanation was suggested that the rate of absorption from the peritoneal cavity gave the circulating immune bodies an opportunity to neutralize, or bind, the antigen before it could reach the cells in sufficient amounts to induce anaphylactic shock. This hypothesis has been put to the test of experiment.

A series of guinea-pigs, ranging about two hundred and fifty grams in weight, were sensitized by the intraperitoneal injection of three-tenths cubic centimeter of the serum of a rabbit immunized against horse serum. The M.S.D. of this serum lay below one-tenth cubic centimeter. Three days after sensitization one of these guinea-pigs received a subcutaneous injection of two-tenths cubic centimeter of horse serum. This animal was killed on the following day and the uterine tracing taken. It revealed the fact that the organ failed to respond to horse serum, and was, therefore, completely desensitized. On the fourth day after sensitization another pig of the series received one-tenth cubic centimeter of horse serum subcutaneously. This animal was tested on the following day in the same manner and was found to be almost completely desensitized. One horn, reproduced in Figure 16, gave a minimal reaction to horse serum; the second horn none. Two other guinea-pigs treated in the same fashion were found to be completely desensitized.

FIGURE 15.—G.P. sensitized by .3 cubic centimeter Serum 435. Three days later .2 cubic centimeter horse serum subcutaneously. Killed on fourth day. No reaction to horse serum. Good reaction to ergamine.

FIGURE 16.—G.P. sensitized by .3 cubic centimeter Serum 435. On fourth day .1 cubic centimeter horse serum subcutaneously. Killed on fifth day. Reaction to .1 cubic centimeter of horse serum delayed three minutes, and very sluggish. Reaction to ergamine good.

FIGURE 17.—G.P. sensitized by .3 cubic centimeter Serum 435. On fourth day 2.5 cubic centimeters Serum 206 intravenously, followed by .2 cubic centimeter of horse serum subcutaneously. Serum 206 was that of a rabbit highly immunized against horse serum. G.P. killed on fifth day.

FIGURE 18.—G.P. sensitized by .3 cubic centimeter Serum 435. On fourth day 3 cubic centimeters Serum 206 intravenously, followed by .2 cubic centimeter of horse serum subcutaneously. Reaction to horse serum prompt, but only moderately strong.

From these observations the conclusion may be drawn that in this series one-tenth cubic centimeter of horse serum given subcutaneously desensitizes the uterine cells.

In another group of guinea-pigs of the same series the subcutaneous desensitizing injection of two-tenths cubic centimeter of horse serum was immediately preceded by an intravenous injection of either two and one-half or three cubic centimeters of the same immune serum as had been used for sensitization. These guinea-pigs were exsanguinated on the following day and uterine tracings were taken. In every instance it was found that the addition of horse serum produced a distinct contraction. Tracings from two different animals are reproduced in Figures 17 an 18. These tracisgs show that the uteri still contain small amounts of free antibody. The contraction shown in Figure 18 is neither so prompt nor so energetic as is found in sensitized controls, showing that a certain amount of desensitization had taken place. On the other hand, it is also evident that the presence of a large excess of free antibody in the serum served to protect the cellular antibodies of these animals in considerable measure, inasmuch as there is a distinctly lower degree of cellular saturation than in the controls, as shown in Figures 15 and 16. Thus it is safe to conclude that circulating antibody protects fixed antibody against saturation by antigen. This action becomes especially manifest where the antigen is introduced subcutaneously, and is therefore only slowly taken up into the circulation. Even in case the antigen is introduced intravenously, the circulating antibody exercises a certain degree of protection, but in that case this function is largely obscured, as has previously been shown, by the increased avidity of the fixed, or cellular, antibody. The serum obtained from the animals in this set of experiments was in each case injected into a normal guinea-pig intraperitoneally, and the latter were tested two days later by the intravenous injection of horse serum. In no instance did these animals manifest any anaphylactic symptoms. Thus it is evident that the gradual absorption of the antigen had served to neutralize the major portion of the circulating antibody within the twenty-four-hour interval following the desensitizing injection.

Intermediate in position between the intravenous and the subcutaneous introduction of antigen would lie the peritoneal route. Owing to the exigencies of the method, it has not been possible

to experiment with this route. The intraperitoneal injection of antigen would act locally on the uterine antibody, and would desensitize that organ long before there was any general or constitutional desensitization of the body as a whole. The uterine preparation would, therefore, give an entirely false impression regarding the saturation of these cellular receptors which actually play a much more important part in the induction of the anaphylactic symptom-complex, for example, of the bronchial musculature. This detail of technic is one which must always be kept in mind in performing experiments which are designed to accept the uterine reaction as representative of that of the other cells of the body. Naturally, this purpose can only be accomplished by placing the uterine cells on a par with all the other cells of the body, a precaution which necessarily excludes the use of the peritoneal route.

It is, however, permissible to reason out the effect of an intraperitoneal injection, judging thereof by the effects obtained after the intravenous and the subcutaneous injection of antigen. The absorption from the peritoneum is vastly more rapid than that from the subcutaneous tissue, as is shown by the fact that anti-anaphylaxis is developed much more rapidly by the former route. Hence it is fair to conclude that even in the presence of considerable amounts of free antibody the increased avidity of the cellular receptors would permit them to anchor a certain amount of antigen, even where relatively minute amounts of this substance are introduced. At the same time not enough antigen would be present to saturate the free antibody. This combination satisfactorily explains the observation previously noted in this paper in connection with the intravenous injection of antigen, that a guinea-pig may be anti-anaphylactic by virtue of the previous administration of antigen, while the blood still contains sufficient antibody to sensitize a normal guinea-pig passively. I have, however, never seen such a condition resulting from the subcutaneous injection of antigen, and from the data obtained in these experiments it seems doubtful whether this effect could be produced by that mode of administration.

An accessory point of interest is the observation, illustrated by the preceding experiments, that the same amount of circulating antibody is much more effectively saturated by the identical dose of antigen when given subcutaneously than when given in-

travenously. It is possible that the time interval, which differed by almost twenty-four hours, played a part in this changed effect. It is also possible that the difference presents a vital analogy with the well-known Dungern-Danyz phenomenon in the test-tube. At the present time these explanations must be regarded merely as possible alternatives, the question not having been experimentally approached.

Conclusions. — 1. The observations made in a previous paper are verified, namely, that circulating antibody protects a sensitized animal to a certain extent from the anaphylacitc effect of an intravenous injection of antigen. Furthermore, that the degree of protection is not quantitatively proportional to the amount of antibody in circulation, but is considerably less than this.

2. The uterine preparation, which is taken to exemplify the condition of all the cells of the body, may be desensitized by the intravenous injection of antigen, whereas the circulating antibody of the same guinea-pig remains unsaturated, as is evidenced by the fact that the blood effectively sensitizes a normal guinea-pig.

3. The conclusion is drawn that the avidity of cellular antibody for antigen is considerably greater than that of circulating antibody.

4. If sensitized guinea-pigs are previously given large amounts of antibody intravenously their cells are not desensitized by an amount of antigen, given subcutaneously, which suffices completely to desensitize the control animals. At the same time their circulating antibodies are saturated to such an extent as to preclude the possibility of passively sensitizing normal guinea-pigs with their blood.

5. The conclusion is drawn that circulating antibody protects fixed antibody from antigen, when the latter is introduced by a route permitting of slow absorption. It seems evident that in the usual processes of disease this mode of protection must be in some degree effective.

6. These facts explain the observation that immunized guinea-pigs may be killed by antigen when administered in large amounts intravenously. Furthermore, they explain the fact that an anti-anaphylactic guinea-pig may contain in its serum sufficient antibody passively to sensitize a normal guinea-pig.

IX. The relation between partial desensitization and the minimal lethal dose in anaphylaxis.

In a previous paper I have laid stress on the fact that a sensitized guinea-pig which has received a dose of antigen insufficient to induce complete anti-anaphylaxis is still demonstrably altered thereby. Such an animal can no longer be killed by a dose of antigen which suffices to produce anaphylactic death in the control animal sensitized by the same dose of immune serum. It can, however, be killed if the antigen be administered in considerably larger amounts. This fact is of such importance in the further development of the present study that the original table is herewith produced:

Table.

The animals of this series were passively sensitized with 1.5 cubic centimeters of S. 204, from a rabbit highly immunized against horse serum. The minimal fatal anaphylactic dose is .005 cubic centimeter of horse serum given intravenously.

G.P.	1st Desensitizing Injection.	2d Desensitizing Injection.	Anaphylactic Injection.	Result.
1	2d day, 0.001 cc. H.S., i.p.	2d day, 1 hour later, 0.005 cc. H.S., i.p.	3d day, 0.05 cc. H.S., i.v.	†
2	" " 0.001 " " "	2d day, 1 hour later, 0.005 cc. H.S., i.p.	3d day, 0.05 cc. H.S., i.v.	†
3	" " 0.01 " " "	3d day, 0.005 cc. H.S., i.v. *Convulsions.*	3d day, after 15 min., 0.2 cc. H.S., i.v.	†
4	" " 0.01 " " "	3d day, 0.01 cc. H.S., i.vi *Severe Symptoms.*	3d day, after 1 hour, 0.2 cc. H.S., i.v.	†

In the present study the same problem has been approached by means of the graphic method. If the uterus of a sensitized guinea-pig, whether actively or passively matters not, be tested according to Dale's method, it is found that the repeated addition of the same amount of antigen produces successive muscular contractions. If relatively small doses of the antigen be used it will be found that quite a series of contractions can be produced, but that eventually they lose their effect and that the uterus finally fails to contract. If now a much larger amount of antigen be added it will be found, unless the antibody has been entirely exhausted by the preceding manipulation, that a contraction can be elicited.

FIGURE 19.—G.P. actively sensitized by .01 cubic centimeter of horse serum subcutaneously. Nineteen days later, uterus tested. The successive additions of .01 cubic centimeter of horse serum produce first a moderate contraction, second a maximal contraction, third a small contraction. The final addition of .2 cubic centimeter of horse serum produces a maximal contraction.

The same result is obtained with the uteri of animals immunized by repeated large injections, as shown in Figure 20.

These tracings show that a uterus which could no longer be stimulated by one-hundredth cubic centimeter of horse serum still responded well upon the addition of two-tenths. This observation, which is quite in harmony with that previously described for the living animals, leads to the conclusion that partial desensitization of the uterus increases the M.A.D.(D.) (minimal anaphylactic dose of Dale's method).

The same result has been reached by a somewhat different method. Guinea-pigs were passively sensitized by large amounts of the serum of rabbits immunized against horse serum. These animals then received a subcutaneous injection of an amount of horse serum insufficient completely to desensitize them. On the following day the uteri were removed and tested by Dale. The result showed that the M.A.D.(D.) was very much higher than in control animals which had received the same sensitizing dose but no desensitizing injection. This result is illustrated in Figure 21.

FIGURE 20.—G.P. actively immunized by the subcutaneous injection of 1 cubic centimeter on three successive days. Uterus tested on eighteenth day. The results are the same as in the preceding tracing.

FIGURE 21.—G.P. sensitized passively by 1 cubic centimeter of serum of rabbit vs. horse serum. On following day given .01 cubic centimeter of horse serum subcutaneously. On third day, uterus tested. There is no reaction to one ten-thousandth of horse serum; the reaction to one one-thousandth is sluggish. These results when compared with Figures 5-8 show marked desensitization.

FIGURE 22.—G.P. actively sensitized by the intraperitoneal administration of .01 cubic centimeter of horse serum. On the twenty-sixth day .01 cubic centimeter horse serum subcutaneously. On twenty-seventh day, uterine tracing. A good contraction results from the addition of one ten-thousandth of horse serum, indicating a very mild degree of desensitization.

FIGURE 23.—Same preparation as in preceding figure, except that .1 cubic centimeter of horse serum was given to desensitize. The contraction to .1 cubic centimeter of horse serum is very sluggish, indicating a marked degree of desensitization.

The pig in this instance had received one cubic centimeter, five M.S.D. of immune rabbit's serum. On the following day it received one-hundredth (.01) cubic centimeter of horse serum subcutaneously. On the third day the uterus was tested. The M.A.D.(D) was found to be one-thousandth cubic centimeter for one horn of the uterus; it was one-twentieth for the other. Such differences between opposite cornua are not infrequently seen where antigen is given to a sensitized animal, and argue for its unequal distribution in the body. If this result be compared with the controls described in the seventh study it will be clear that the M.A.D.(D) has been enormously increased by the intervening desensitizing injection.

The next two figures illustrate the same effect following upon desensitization in actively sensitized animals.

By all three methods of study, therefore, it becomes evident that the anaphylactic state is markedly altered by the addition of sub-lethal amounts of antigen. The interpretation of these facts, however, is not clear. One is, at first thought, tempted to conclude that there is simply a partial saturation of the antibody by the antigen, and that the balance of antibody remaining will naturally require a larger amount of antigen to produce a typical response. Such an interpretation would be in keeping with the results of the preceding study, in which it was shown that there was an inverse relationship between the amount of antibody and the minimal amount of antigen required to produce an anaphylactic response. The conditions, however, do not permit of this explanation, as will be evident from an analysis of the quantitative relations. If it is found that the M.S.D. of a certain immune rabbit's serum is two-tenths of a cubic centimeter, the administration of one-tenth cubic centimeter of this serum will fail to sensitize guinea-pigs even against relatively enormous doses of the antigen, namely, horse serum. Such undersensitized animals cannot be killed by the intravenous administration of even one-half to one cubic centimeter of horse serum. On the other hand, the M.S.D. is effective for amounts of less than one-hundredth cubic centimeter of horse serum. Now, the behavior of partially desensitized pigs is entirely different from this. They do not respond to fractional amounts of the antigen, but are killed in typical fashion by very large amounts. It is impossible to believe that the intermediate injection of antigen has, over a series of

animals, neutralized all of the antibody excepting exactly that amount which may be imagined to lie just below the ascertained M.S.D., and which might be conceived as capable of responding to very large doses of antigen. If the data quoted from the previous paper be examined it will be evident that this condition of partial desensitization, in which a relatively large dose is required to kill, was induced in a fair proportion of the series without any effort or calculation — in fact purely by chance. Such a result is incompatible with the assumption of a quantitative neutralization of all except what might be called the subminimal sensitizing dose of antibody.

By Dale's method the results are analogous. With a little care one can partially desensitize a uterus in such wise that it will fail to respond to one-hundredth cubic centimeter of horse serum, whereas the addition of one-tenth or two-tenths elicits a marked contraction. Now, if such a result is to be interpreted upon the basis of a partial neutralization of the antibody, the strain upon the facts becomes heavier than they can bear. One would be required to believe that by means of an almost careless addition of successive amounts of antigen one had been able to consummate an exquisitely fine degree of partial saturation of antibody, leaving free only that minimal amount which will react only with enormous amounts of antigen.

There is, however, another interpretation of the foregoing data which seems far more reasonable and probable. If it be admitted that the presence of antigen within the cells which contain antibody may modify the reaction of that antibody, the road is open to an understanding of the foregoing phenomena.

In the seventh study it was shown that the preliminary administration of very small amounts of antigen to the uterine preparation induces an increased sensitiveness of the muscle, in virtue of which previously ineffective quantites of antigen become capable of eliciting a contraction. A similar alteration with, however, a reversed effect, has been shown to accompany the preliminary administration of large amounts of antigen. The muscle now fails to contract except upon the addition of very large quantities of antigen. Thus Figures 15 and 16 show that while the second administration of antigen is more effective than the first, the third is markedly less effective than the second. These observations illustrate opposite phases of the same general phe-

nomenon, namely, that the reactivity of the cellular antibody is materially influenced by the presence of antigen.

Upon this theory, it is not the amount of antibody that remains which determines the M.A.D. in a partially desensitized guinea-pig, but the altered character of the antibody. Antibody under these conditions may be supposed to require a large excess of antigen to produce a typical response. The marked increase in the anaphylactic dose after partial desensitization is then simply an expression of this altered reactive condition of antibody in the presence of antigen, and is not explicable upon the basis of a merely quantitative alteration of the antibody.

This theory is supported by the following observation: When a well-sensitized uterus is made to contract by the addition of a small amount of antigen one can produce either one of two final results. If one washes the preparation thoroughly, and then adds fresh antigen in the same amount as before, one can produce a renewed contractile response. If, however, one adds the second dose of antigen, without a previous washing, the contractible response almost invariably fails to occur. This phenomenon is well illustrated by the tracing in Figure 24, which illustrates both modes of procedure. Now the meaning of this fact does not seem to be ambiguous. The presence of the antigen in the surrounding fluid modifies the reactivity of the antibody in the cell in such wise that it fails to react upon the addition of an efficient amount of antigen, and in this way the situation is analogous to that previously postulated in explanation of the lowered reactivity of the partially desensitized cell. The objection may be urged that the conditions compared are not identical. In the latter instance the antigen is outside the cell, in the former it is supposed to be within it. There is as yet no evidence, it may be said, that antigen and antibody can coexist within the uterus, in such wise that the antigen could modify the condition of the antibody. According to current theories of anaphylaxis and immunity it is generally believed that the antigen is at once destroyed by the antibody and that this very destruction, with the formation of toxic decomposition products, is the essential agent of the anaphylactic response.

Such, however, proves to be not the fact. It will be shown in considerable detail in the succeeding study of this paper that antibody can coexist in an intact and unchanged condition within

the same living uterus of the guinea-pig. In the present study this fact will be demonstrated by means of the following experiment: A guinea-pig is actively sensitized, or immunized, against normal rabbit serum. After the lapse of a proper interval the animal is desensitized by means of a special procedure. Instead of receiving an injection of normal rabbit's serum it is given the serum of a rabbit immunized against horse serum. The conditions must be so calculated that the rabbit's serum is fairly strong in order that one may be enabled to administer a large number of antibodies in an amount of rabbit's serum not sufficiently great to produce complete desensitization. This, however, can be accomplished under favorable conditions. The essence of the method consists in the fact that it permits one to identify the antigen in the cells, by virtue of the fact that the antigen is also an antibody, simply through the addition of horse serum. Therefore, in such a muscle preparation, one tests for the autogenous antibody — namely, guinea-pig versus rabbit serum — by the addition of rabbit serum, and for the presence of antigen — namely, rabbit versus horse serum — by the addition of horse serum. The following tracing shows typically the co-existence of these two factors in the same organ, one is almost tempted to say in "the same cell," of the guinea-pig's body.

FIGURE 24.—G.P. actively sensitized by .01 cubic centimeter of horse serum intraperitoneally. On forty-second day, uterine tracing. Uterus heavily overweighted. The lower tracing shows absence of uterine contraction when the horse serum is added in small increments to the Locke's solution. The preparation was then thoroughly washed. The upper tracing shows the effectiveness of the same quantities of antigen when added to fresh Locke's fluid, with the same uterus.

FIGURE 25.—G.P. actively sensitized by .3 cubic centimeter of rabbit's serum intraperitoneally. On sixteenth day 1 cubic centimeter of the serum of a rabbit highly immunized against horse serum is given subcutaneously (S. 435). On the seventeenth day, uterine tracing. The reactivity to rabbit serum is first exhausted. The presence of rabbit antigen is then demonstrated by the reaction induced by the addition of .1 cubic centimeter of horse serum.

FIGURE 26.—G.P. actively sensitized by .4 cubic centimeter of rabbit's serum intraperitoneally. On sixty-third day 2.5 cubic centimeters of serum rabbit versus horse serum (S. 435) is given subcutaneously. Uterine tracing on following day. Antibody and antigen are demonstrated to coexist, as in Figure 25.

FIGURE 27.—G.P. actively sensitized by 1 cubic centimeter of rabbit's serum intraperitoneally. On sixty-third day 2.5 cubic centimeters of serum rabbit versus horse serum (S. 206) is given subcutaneously. On third day thereafter, uterine tracing. The reaction to rabbit serum is very weak, indicating almost complete desensitization. The reaction to horse serum is pronounced, showing the persistence of antigen in the presence of antibody over three days.

FIGURE 28.—G.P. actively sensitized by 2 cubic centimeters of rabbit's serum intraperitoneally. On forty-eighth day, uterine tracing. The reaction to .1 cubic centimeter of horse serum is pronounced, showing the presence of crossed sensitization.

In connection with this experiment there is a technical detail of importance. Guinea-pigs highly sensitized against rabbit serum sometimes react strongly by Dale's Method to horse serum. This is simply an instance of the crossed reactions which not infrequently occur in anaphylaxis. This fact is illustrated in Figure 28.

It is therefore essential in performing the above described experiment to exhaust the reactivity towards rabbit serum before proceeding to test the uterus with horse serum.

The evidence seems, therefore, to be strongly in favor of the theory herein advanced. It has been shown that the presence of antigen in the surrounding fluid lowers the reactivity of the uterus. A fortiori, it would be expected that the presence of antigen in the cell would produce the same effect. It has been demonstrated that antigen does exist in unaltered form in the uterus of partially desensitized guinea-pigs. There can, therefore, be no reason to doubt that the lowered reactivity of the partially desensitized guinea-pig, as well as of the partially desensitized uterus, is due to the presence of this antigen.

The condition of an organism in which antigen and anti-body coexist is not uniform. Two tracings in this paper taken from opposite horns of the uterus show that there may be a marked difference in the balance between these two factors in various parts of the body. There is a steady tendency towards the reduction of the antigen, as will be shown in the following paper, and towards the relative increase of antibody in the cells. There is correspondingly a well attested phenomenon, namely, the gradual displacement of a condition of desensitization by a renewal of the condition of hypersensitization.

It is of interest in this connection to note the fact that complete desensitization is also to be explained upon the same basis. Thus, if a sensitized guinea-pig receives a subcutaneous injection of antigen this protein reaches the cells only very slowly and in gradually increasing amounts. It is evident that each minute increment which reaches the cell fortifies it against the next succeeding large amount, and thus the animal is desensitized without ever undergoing a typical anaphylactic reaction. The same process may be imitated by Dale's method, as shown in Figure 29.

FIGURE 29.—G.P. passively sensitized by the intraperitoneal injection of .2 cubic centimeter of serum rabbit versus horse serum (S. 206). Uterine tracing two days later. The tracing illustrates the desensitization of the uterus by the gradual increase of antigen in the surrounding fluid. It illustrates the mechanism of desensitization in vivo by the subcutaneous injection of antigen.

The opposite horn of this uterus is shown in Figure 30 as a control.

FIGURE 30.—Opposite cornu of same uterus, to show normal reactivity of the organ.

Conclusions. — 1. An isolated uterus which has been partially desensitized by the addition of small amounts of antigen fails to respond except upon the administration of very large amounts of antigen.

2. The same condition holds true of the living animal. A guinea-pig which has been partially desensitized by the injection of antigen can be killed only by the use of much larger quantities than are required for the unaltered sensitized control animals.

3. This phenomenon cannot be explained upon the theory of incomplete saturation of the cellular antibody.

4. The explanation is advanced that the increased dose of antigen necessary to elicit an anaphylactic response is due to an altered condition of the antibody. It is suggested that the presence of antigen depresses the reactivity of the antibody in the cells, necessitating the use of much larger amounts in order to produce a response.

5. The probability of this theory is illustrated by the fact that the presence of antigen in the fluid surrounding the muscle preparation in Dale's method markedly reduces the reactivity of the cells to antigen.

6. Further, as is shown by means of a special technic, the uterus of a partially desensitized guinea-pig contains unaltered, intact antigen at the same time that it contains an amount of antibody capable of reacting well upon the addition of fresh antigen in considerable quantity.

7. The most striking characteristic of partial desensitization, therefore, namely, the marked increase in the minimal anaphylactic dose, is attributed to the presence of such amounts of antigen in the cells as serve to lower the activity of the antibody.

8. Complete desensitization is shown to depend upon the same general process, with the difference that the reactivity of the antibody is entirely abolished by a great excess of antigen.

X. The persistence of intracellular antigen as a factor in immunity.

The preceding study has shown that the presence of antigen in the cell is an important factor in the desensitization of the guinea-pig. In the present study the same factor has been studied from the standpoint of its bearing upon immunity. In this connection the word immunity is used in the current sense as contrasted with hypersusceptibility, although, as I have attempted to show in a previous paper, no hard and fast line actually separates these two conditions. A guinea-pig which has received a preliminary injection of .01 cubic centimeter of alien serum becomes typically hypersensitive and can be killed by the intravenous injection of .01 cubic centimeter, or less, of that serum. If, however, the preliminary injection consists of a large amount of the foreign serum, as three cubic centimeters (an "ictus immuni-satorius," in Ehrlich's term), the guinea-pig becomes hypersensitize to a much less marked degree, so that more than two-tenths cubic centimeter is required to kill it. From the standpoint of intraperitoneal injection, however, the difference of degree apparently becomes altered to a difference in the mode of reaction; the first guinea-pig can be killed with anaphylactic symptoms by means of the alien serum, whereas the second pig cannot, and is, therefore, apparently immune. Coincident with these differences in the reaction to the reinjection of antigen are certain other differences, involving the period of incubation, the size of the minimal desensitizing injection, and, most especially, the number of anti-

bodies in circulation. In the immune pig the latter are usually present in considerably greater amount than in the typically hypersensitive animal. It is upon this latter fact that stress has been laid in the past, in attempting to explain the greater resistance of the immune pig. The circulating immune bodies are supposed to bind the antigen, and so to protect the cells. Although this may be a factor in the result, careful quantitative study discloses the fact that in many guinea-pigs prepared by massive preliminary injections the blood is not nearly rich enough in antibody to account for the increased amount of antigen required to produce anaphylactic death. In the present study the attempt has been made to determine experimentally whether the persistence of antigen in the cell may not be a factor which also contributes materially to the resistance of animals prepared by means of massive doses of antigen.

The procedure has been essentially similar to that described in the preceding study, using as antigen a foreign serum rich in antibodies. Three guinea-pigs were given an intraperitoneal injection of three cubic centimeters of the serum of a rabbit immunized against horse serum. The minimal sensitizing dose of this serum was two-tenths cubic centimeter. The first of these animals was exsanguinated on the eleventh day after sensitization. The serum was injected intraperitoneally into a normal guinea-pig, 1a. The uterus was at once removed from the first animal and tracings taken. The uterus was tested for its reactions, first to rabbit serum, then to horse serum. The results shown in Figure 31 demonstrate that the pig had at this time developed a very moderate sensitization towards rabbit serum. At the same time the reaction to horse serum reveals the persistence of the rabbit antibodies.

In this connection there is a technical detail which requires notice. The uterine tracings show only a moderate reaction to rabbit serum at a time when the living animals would, according to experience, be killed by an intravenous injection of the rabbit serum. This discrepancy is due to the fact that the quantitative relationships are different, owing to the technical requirements of the Dale experiment. In vivo, guinea-pigs sensitized by massive doses require often as much as four-tenths cubic centimeter of the antigen to produce anaphylactic death. This amount is diluted by perhaps ten to fifteen cubic centimeters of circulating blood, thus the antigen must be present in a concentration approximately of one in thirty. In the Dale experiment, however, one-tenth cubic centi-

meter is introduced into one hundred and fifty cubic centimeters of Locke's fluid, giving a dilution of one in fifteen hundred. It is not advisable to use large amounts of rabbit serum in making these tests, since the normal uterus occasionally reacts to the presence of high concentrations of alien serum. The difference in concentration, however, amply accounts for the difference in result.

FIGURE 31.—G.P. sensitized by 3 cubic centimeters Serum R. vs. H.S. Tested on eleventh day. Reaction to .1 cubic centimeter horse serum maximal; to .1 cubic centimeter rabbit serum, weak.

Guinea-pig 1a, passively sensitized with the serum of the animal, was given an intravenous injection of three-tenths cubic centimeter of horse serum on the third day after sensitization, and reacted with violent symptoms, but did not die. Therefore, the conclusion is drawn that within eleven days after sensitization by a large dose of alien serum a guinea-pig has free antibodies thereto in the blood, and fixed antibodies in the cells; at the same time the antigen itself persists in the blood.

The second guinea-pig (G. P. 2) was exsanguinated on the thirteenth day after injection. Its serum was given to Guinea-pig 2a, which was later tested by the intravenous administration of horse serum and died in convulsions. The uterus of Guinea-pig 2 was tested against both rabbit and horse serum. The accompanying figure demonstrates both the presence of antibody by the reaction to rabbit serum and the persistence of antigen by the reaction to horse serum.

FIGURE 32.—G. P. sensitized by 3 cubic centimeters Serum R. vs. H.S. Tested on thirteenth day. Reactions to horse serum and to rabbit serum about equal.

The third guinea-pig was treated in the same fashion on the fifteenth day after injection. The serum effectively sensitized a normal guinea-pig so that death ensued upon the injection of horse serum. The two horns of the uterus reacted both to rabbit serum and to horse serum, but in a somewhat different fashion. The first horn, shown in Figure 33, presents practically no reaction to horse serum, whereas there are two good reactions to .01 cubic centimeter of rabbit serum.

The second horn presented a good reaction to .01 cubic centimeter of rabbit serum.

On the first horn of this animal, therefore, the antibodies to rabbit serum were present in such excess as to abolish the re-

action to horse serum. In the second horn, however, there was still sufficient antigen to permit of a good response to horse serum. This unequal distribution of equilibrium between the two factors is, as has been previously stated, a frequently observed phenomenon.

A comparison of the uterine reactions to rabbit serum and to horse serum in this series of animals indicates a progressive change. The reaction to rabbit serum becomes increasingly more pronounced. That to horse serum becomes steadily less marked. Thus it is clear that there is an accumulation of antibodies against rabbit serum, the antigen and, coincidently, a neutralization of the antigen itself. The antigen has been shown to persist in the cells over a period of at least fifteen days. It must be remembered, in this connection, that the presence of the reaction to horse serum is by no means to be regarded as a delicate reagent for the antigen, inasmuch as two-tenths cubic centimeter of the rabbit serum was required for sensitization. It is, therefore, entirely reasonable to suppose that traces of rabbit serum might persists in the cells for a considerably longer period, although not in sufficient amount to permit of a reaction to horse serum by the uterine preparation. Finally, it has been shown that the blood of the animal contains free antibody during this entire period, simultaneously with the presence of antigen in the body cells.

FIGURE 33.—G.P. sensitized by 3 cubic centimeters Serum R. vs. H. S. Tested on fifteenth day. No reaction to horse serum. Sharp reactions to rabbit serum.

The results described for this series have been duplicated in two other series of animals similarly prepared, showing their general validity. It is possible to demonstrate the same fact in vivo, by injecting duplicate animals of such a series on the same day, the one with horse serum and the other with rabbit serum. Both animals present anaphylactic symptoms.

Conclusions. — 1. When a guinea-pig is sensitized by the intraperitoneal injection of a large amount of the serum of a rabbit immunized against horse serum it becomes possible by Dale's method to determine the persistence of sensitization to horse serum over a period of fifteen days. The degree of sensitization gradually diminishes.

2. At the same time there is a gradual development of sensitization towards rabbit serum.

3. Coincidently, the presence of circulating antibodies towards horse serum is demonstrated in the serum by using it to passively sensitize normal guinea-pigs.

4. These data are interpreted to indicate that both antigen (rabbit's serum), and antibody thereto, coexist for some time in the cells of a guinea-pig immunized by large doses of foreign serum; and that the blood at the same time contains free antigen.

5. The suggestion is made that the persistence of antigen in the cell is a factor which contributes materially to the resistance of animals sensitized by massive doses of antigen.

XI. The share of intracellular antigen in immunity and in desensitization. Theoretical considerations.

The experimental data which have been presented in the preceding studies have a not unimportant bearing upon certain of the phenomena of anaphylaxis and of immunity. It has generally been assumed in the past that the function of circulating antibody is protective in nature. Those who accept the humoral theory of anaphylaxis, notably Friedberger, believe that an excess of free antibody in the serum leads to a rapid degradation of the introduced antigen, with the production of non-toxic end-products. I am not here immediately concerned with that view, believing that there is more ground for the acceptance of the cellular theory, which maintains that the anaphylactic reaction invariably occurs within the cell. In outlining the general features of the cellular theory I have shown in previous publications that immunized animals are also potentially anaphylactic. Such animals, however, differ from animals treated by means of minute doses of alien serum in the important particular that a far larger dose of antigen is required to kill them. It seemed probable that this quantitative difference could be explained upon the basis of the protective action of the circulating antibodies. It was shown that animals prepared by means of large doses of alien serum do, as an actual fact, frequently contain many more antibodies in their blood than do the typically hypersensitive animals, which have been injected with minute amounts of foreign serum. This interpretation is in harmony with the traditional views concerning the

protective function of free antibody. Careful analysis of this explanation, however, proves that it is not in keeping with the observed facts. In the first place, it is not infrequently found that the immunized animal contains an amount of circulating body which is entirely insufficient to explain the degree of immunity which it displays. Such an animal may have in its blood not more than two or three sensitizing units of antibody, although in other instances the amount may be equal to even twenty or thirty such units. On the other hand, this group of animals is regularly refractory to anything less than very large amounts of antigen given intravenously. In a series of guinea-pigs prepared by the injection of two cubic centimeters of horse serum upon three successive days the M.A.D. was found to be four-tenths cubic centimeter of horse serum. For guinea-pigs prepared by a single injection of .01 cubic centimeter of horse serum, on the other hand, the M.A.D. did not exceed .05 cubic centimeter. The former group, therefore, were refractory towards more than seven M.A.D. of antigen, as determined in the latter group. If this quantitative difference is to be explained upon the basis of the increased amount of circulating antibody it is clear that very large amounts of the latter must be present. Judging by the results of previous experiments it is reasonable to expect that the blood would have to contain as much as thirty or forty sensitizing doses. Such, however, proves in the majority of the animals tested to be not the case. In this essential particular the theory is entirely inadequate to explain the facts. It has been shown in this paper that the presence of antigen in the cells very materially modifies the reactivity of the cellular antibody. The theory is suggested that the relative immunity enjoyed by animals prepared by means of massive injections of antigen depends in major part upon the persistence of traces of this antigen in the cells. That antigen does persist in the manner postulated has been demonstrated. That it markedly lowers the reactivity of the cellular antibody has also been demonstrated. It seems impossible to doubt, therefore, that it is the effective agent in producing their resistance to the anaphylactic effect of antigen.

This theory, however, does not entirely dispose of the protective rôle of the circulating immune body. It has been shown that the antibody of the blood does, in fact, very materially protect the fixed, or cellular, antibody against antigen when the latter

is introduced by the subcutaneous route. The gradual access thus afforded the antigen to the cellular antibody must necessarily gradually desensitize the cells, and so prevent anaphylactic shock. In spite of the presence of a great excess of free antibody the superior avidity of cellular antibody is such as to insure its partial combination with antigen, even when subcutaneously introduced. In all probability this represents the mechanism of protection of the cells in most of the natural processes of disease.

There are certain facts which are not as yet adequately explained by the data hitherto presented. In a previous publication it was shown that the immunized animal, prepared by the injection of a massive dose of antigen, requires a very large dose of antigen for the production of desensitization. It might be assumed that this fact argues in favor of the presence of a great access of antibody in the cells. Such an interpretation would be in harmony with the rapid development of the anaphylactic condition in animals so prepared, and with the presence of a large number of antibodies in the circulating blood. Moreover, it offers an exact analogue to the conditions observed in passive sensitization, where it is found that guinea-pigs which have received the larger sensitizing doses require larger amounts of antigen to desensitize. Upon this hypothesis it might be assumed that the antibodies present in the cells, although present in very great access, do not unite with introduced antigen except when present in very large amounts in the circulating blood. For many reasons this view is unsatisfactory. The tracings show that the guinea-pig uteri, at the time when the living animals require as much as .2 cubic centimeter to desensitize them, have a very limited capacity of response to antigen, showing a very limited supply of antibody. Thus, it is inconceivable that an animal with this available amount of antibody should require such relatively enormous quantities of antigen to partially satisfy its affinity for antigen, and thereby render it refractory to the addition of fresh amounts. There is, however, another possible explanation, which seems to be more in keeping with the facts. It may be assumed that the coexistence of antigen and antibody in the cells establishes a condition of equilibrium. In this condition, wherein the affinity of antibody for antigen is largely satisfied, there is, as has been shown, a marked diminution of the reaction energy of the antibody towards fresh, or additional, antigen. It may be assumed, therefore, that

antibody in this condition fails to respond to fresh antigen unless it is presented in very large amounts. If the antigen is introduced in such amounts that its concentration in the surrounding fluids of the body, blood or lymph, falls below a certain level, it fails not only to stimulate the reactivity of the cellular antibody, but fails absolutely to accomplish any union therewith. This is demonstrated by an experimentally observed fact, the details of which are to be found in Table II. of Study V. of this series of papers; it is there seen that the addition of antigen in amounts less than two-tenths cubic centimeter not only fails to desensitize guinea-pigs prepared by massive doses, but fails to lower in any degree the reactivity of the animals to the eventual lethal dose of antigen. Thus, the lethal dose for the immunized controls (4 and 5) of the series was found to be four-tenths cubic centimeter of horse serum. Animals which had received desensitizing injections of one-tenth and two-tenths cubic centimeter (8 and 16) were killed by amounts of seven-tenths and of one cubic centimeter of antigen. Had the previous desensitizing doses of antigen been bound by the cells the reactivity of the cellular antibody would inevitably have been reduced to a much lower grade than is indicated by the observed facts. Thus the view is justified that the antibody of the cells, in the presence of antigen in the cells, is in a condition of fairly stable equilibrium, in which it fails to bind additional antigen unless the latter be present in relatively high concentration in the surrounding fluid. In relatively low concentration the fresh antigen fails to disturb the intracellular equilibrium and remains in the fluids entirely without effect upon the cellular antibody. In high concentration, however, it upsets the cellular equilibrium and unites with the cellular antibody, producing a characteristic cellular discharge with desensitization of the animal. What proportions of antigen and antibody in the cell produce a stable equilibrium is a matter for future research. It is tempting to suggest that the concentration of extracellular antigen must exceed that of intracellular antigen before the cellular antibody can react with the former. It is tempting to draw an analogy with the laws of mass action in physical chemistry. These analogies, indeed, are so evident that they hardly require further elucidation. In the absence, however, of quantitative relationships it seems wiser to accept the facts in their present form

and to leave the determination of the quantitative relations to future investigation.

As regards the process of desensitization, either partial or complete, it has already been pointed out that an important factor is the introduction of antigen into the cell. In this connection, again, there are certain observations which require further elucidation. It was shown by Weil and Coca that passively sensitized animals, which had been desensitized by an injection of antigen, could be passively resensitized by a renewed injection of alien immune serum. One must assume in this case that the cells after desensitization contain a combination of antibody and of antigen, and that they then take up the antibody introduced in the resensitizing injection. Experiment shows that animals resensitized in this fashion react exactly as do the primary sensitized controls; in other words, the sensitizing dose corresponds almost accurately with the resensitizing dose. This observation does not appear to fall in line with the theory that the presence of antigen in the cell materially affects the reactivity of antibody for, in accordance with that theory, it might be expected that the reactivity of the freshly introduced antibody would be materially lowered and that its effectiveness would be proportionately diminished. As has been said, the facts fail to sustain this expectation. It is possible, however, to offer a tentative explanation which does not do violence to the theory of antibody — antigen equilibrium. It must be assumed, in accordance with what has already been demonstrated, that the intracellular combination of antibody and antigen is relatively firm and stable. If this is the case it is quite conceivable that the cell may absorb fresh antibody, and that this antibody may exist in a free and active state in the cellular tissue in spite of the co-existence of a stable combination of antigen and antibody, previously bound, in the same cellular tissue. Indeed, one might almost expect, à priori, that such would be the case. If one were to visualize these conditions in terms of the well-known conceptions of Ehrlich, one would represent a certain number of the rabbit-serum receptors of the guinea-pig cells as occupied by rabbit serum upon the first sensitizing injection. These rabbit-serum elements then become saturated with horse serum, for which they possess specific side-chains, upon desensitization. Finally, upon resensitization, a certain number of the unoccupied rabbit-serum receptors of the guinea-pig cells again anchor rab-

bit-serum, thereby resensitizing the organism toward horse serum. Such a picture would present no point of contact between the freshly introduced antibody and the previously bound antigen.

Upon this theory it is plain that the reactivity of the previously introduced antibody must be abolished by its union with antigen, whereas the freshly introduced antibody, which has not entered into any combination with antigen, retains an unaltered condition of reactivity. If, on the other hand, the intermediate injection had introduced very large amounts of antigen, it is practically certain that resensitization would fail, inasmuch as free antigen would be present in sufficient amounts to bind the fresh antibody.

The bearing of the facts adduced in this paper upon the question of antisensitization is quite apparent. It is evident that antigen is gradually destroyed in the animal, and that this process is necessarily more rapid and more effective in animals already highly, or even imperfectly, immunized by previous injections of the same antigen.

There is another fact of considerable interest which may perhaps find an explanation in the theory that antigen in the cell modifies the reactivity of antibody. In Study V. of this series it was shown that the minimal anaphylactic dose of antigen after passive sensitization against horse serum is as low as .005 cubic centimeter. After active sensitization by the preliminary injection of one-tenth cubic centimeter of horse serum the M.A.D. lies between .02 and .05 cubic centimeter. It is perhaps possible to attribute this difference to the persistence of traces of antigen in the cells after active sensitization. Such an explanation is in harmony with the fact, already explained upon this basis, that massive doses result in an anaphylactic condition requiring a still higher M.A.D. In passive sensitization, of course, one must suppose that antibody alone is introduced, which excludes the disturbing effects of the antigen in the cells.

So much stress has been laid upon the protective value of antigen in the cell that it seems advisable at this point to call attention again to the fact that the circulating antibody does unquestionably confer a certain degree of protection upon the cellular antibody, acting as Weigert has said like the lightning-rod. It is true that this function is not advantageously displayed when antigen is introduced intravenously, but unquestionably it is effective when the antigen is introduced slowly, as by the subcu-

taneous route. Thus in the usual processes of disease it must play an important rôle.

Attention must be drawn to a factor in immunity which has been emphasized by the work of other observers. It has been shown that traces of antigen may persist in the circulating blood of an immunized animal in the presence of large amounts of antibody. Thus precipitin and precipitinogin may be demonstrated in the blood of the same animal. Therefore, an animal which has received one injection or repeated injections of antigen may be assumed under certain conditions to possess: 1, cellular antigen; 2, cellular antibody; 3, circulating antigen;; 4, circulating antibody.

The bearing of each of these factors has been considered with the exception of that of the circulating antigen. From a consideration of the facts discussed in connection with Figure 24, it is evident that this is an element which is a factor, inasmuch as it modifies the reactivity of the cells towards freshly introduced antigen.

Upon the relative proportions of these four factors, therefore, will depend the reaction of the animal. The tendency, of course, is progressively in the direction of an abolition of antigen and of an accumulation of antibody.

One fact must always be kept in mind in judging of the data presented in this paper. The demonstration of antigen in the cells by means of the special technic which employs immune serum as antigen is in a way a very loose determination. Only relatively large amounts can be demonstrated by this method, since it fails to reveal the presence of amounts which lie below the reactive dose. It must be assumed, therefore, that antigen persists in smaller amounts for a period of time considerably in excess of that which has been demonstrated. It is to be hoped that refinements of technic will permit of the detection of these residual amounts of antigen.

Finally, it is hardly necessary to add that the mechanism of anaphylaxis and immunity herein described may or may not have universal validity throughout the animal kingdom. The experiments have been made upon guinea-pigs and the results hold for that species. Whether or not all other types of the animal kingdom fall in the same category is a question which accumulated observation alone can adequately determine.

The conclusions for Study VII. are to be found on page 10; those for Study VIII., on page 18; for Study IX., on page 26; for Study X., on page 31.

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PRECANCEROUS DISEASES AND PRECANCEROUS LESIONS, ESPECIALLY IN THE BREAST.*

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WHENCE and how does cancer develop? The two queries cover the subjects of the formal and the causal genesis of the disease. The formal genesis of cancer is a morphological study which traces the fully developed tumor to the cells of origin. The causal genesis is a physiological subject and deals with the factors which bring about the tissue changes observed.

Until the sources and developmental stages of cancer are rather fully traced the study of causal genesis must proceed under difficulties. Hence for many years minute attention has been given to the very earliest stages of carcinoma and no diagnosis of tumors can be regarded as satisfactory unless the exact cells of origin can be stated.

The formal genesis of a large class of neoplasms was disposed of by Cohnheim, and by many others before and after him, who traced the beginnings of tumors in congenitally misplaced and often em-

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bryonal cell groups. While it is still impossible to state how far the principle of Cohnheim's theory applies to tumor genesis, the comprehensive list of observed tissue abnormalities predisposing to tumor growth as catalogued by R. Meyer, R. Williams, and contributed by many others, far exceeds anything Cohnheim could have postulated. The theory may also be made to include structural abnormalities or tissue predispositions which are not revealed by obvious microscopical changes, but which there is strong reason to believe exist.

On the other hand, clinical observation has long asserted that the great majority of important tumors are not determined by congenital abnormalities in tissue structure, but arise from once normal but previously altered tissue cells, and that various forms of chronic inflammation are observed to precede the development of most tumors.

Thus Billroth observed that cancer almost never arises in a normal breast. v. Brunn, among 368 cases of cutaneous cancer of the limbs, found only 48 in which no previous alteration in the tissues could be demonstrated. v. Bergmann stated that primary cancer of the extremities without previous changes in the skin does not exist. In the various organs throughout the body it is universally recognized that certain pathological conditions are followed in a variable but high proportion of cases by carcinoma. For these conditions Orth employs the term "precancerous diseases," but it should be emphasized that these diseases possess in themselves not a single essential element of the cancerous process. They are merely observed to precede and favor the development of cancer.

A glance at some of these diseases serves to show how intimate is the connection between the

so-called precancerous disease and the tumor process, and under what conditions the one follows the other.

Cutaneous Cancer.—In the skin Darier recognizes three distinct precancerous conditions: (1) Malformations, (2) non-inflammatory degenerations, and (3) inflammatory processes and their sequels.

(1) The chief malformation leading to cancer is the nævus, which becomes malignant in a small proportion of cases and under conditions which have not been clearly defined. Darier has treated them with many destructive agents and never saw cancer develop from the irritation. Yet the case of the nævus is distinctly different from that of the true precancerous lesion and might better be considered in a separate category. Melanoma springs fullfledged from the focus of origin without the long preliminary changes observed in strictly precancerous lesions.

(2) Senile and presenile degeneration of the skin takes the general form of keratosis, and is seen in the seaman's skin, and other disorders leading to multiple cancer. It affects exposed regions subjected to repeated irritation of sunlight, heat, and cold. There is often an hereditary element. There is first hyaline degeneration of the derma, sclerosis of vessels, and atrophy of the Malpighian layer with thickening of the horny layer. Then follow keratosis, scaling, papillary outgrowths, and elongations of deep papillæ. Then very gradually the invasive and destructive features of carcinoma are added. Yet the process in the senile skin is not always the same and Janeway has described as the initial precancerous lesion foci of very large hyperchromatic cells without notable hyperplasia or heterotopia.

In xeroderma pigmentosum there is a family cutaneous dystrophy occurring in infants, marked by dry atrophic, or pigmented, or telangiectatic patches in the skin. After eight to ten years epithelioma resembling the senile type often develops. Arsenical cancer takes much the same course.

(3) Cutaneous cancer of inflammatory origin. In the skin well-known forms of cancer follow in the cicatrical tissue from burns, tuberculous and syphilitic lesions, and amputation wounds. It is characteristic that a long period usually elapses before cancer develops in scars, and that the tumor is very apt to be multicentric or diffuse in origin. The most extensive cancer v. Bergmann ever saw, covering much of the trunk, was in a man of forty years who at four years of age suffered an extensive burn. Many cases, however, develop within five to ten years. The entire area involved in the lesion is usually included in the resulting cancer. Letulle mentions a case of appendical carcinoma arising in an old sclerosed and atrophic organ, and the majority of these cases are preceded by chronic productive inflammation with fibrosis, according to Zaiger 50 per cent., according to Milner 85 per cent. Lupus carcinoma arises in the active lesions of the tuberculous process, but in 30 per cent. of the cases in the scar tissue (Steinhauser). The fact that it comes about the thirtieth year of the disease and never before the fifth shows that considerable cicatrization is an essential preliminary. Several cases of carcinoma in syphilitic scars are reported by Ashihara.

X-ray dermatitis shows a remarkable tendency to develop carcinoma through a process in which cicatrization is an essential factor. A period of three to eleven years is required for the appearance

of the malignant process, which is preceded by necrosis of the tissue, occlusion of blood and lymph vessels, fibrosis, and overgrowth of epithelium (Porter, Wolbach). In the more chronic cases fibrosis and atrophy predominate and the lesion resembles premature senescence (Lindhorn).

The scarring of ulcers is a well-known condition predisposing to cancer. A familiar example is the eczematous and varicose ulcer of the leg which if given time occasionally results in epithelioma.

The relation of gastric ulcer to cancer still remains to be defined. Among recent writers Hirschfeld places the proportion of ulcers that become cancerous at 5 to 6 per cent., and summarizes clinical and statistical data indicating that the proportion is small. Boekelmann collects a series of estimates varying from 3 to 5 per cent. In Vienna 2 per cent. of the subjects coming to autopsy show scars of healed ulcers. In France opinions are widely at variance. Wilson and McCarty from the Mayo clinic report that 68 per cent. of their gastric ulcers were complicated by carcinoma. In my own material, both surgical and post mortem, the proportion is much less, and I sometimes find it difficult to distinguish between ulcerating cancer and cancerous ulcers. In no field is the minute analysis of the histological changes preceding cancer more difficult or important. As a rule cancer develops only in ulcers of long standing and after a preliminary period of multiple adenomatous growths on the margins of the ulcer.

Cancer Resulting from Chronic Productive Inflammation of Glands or Mucous Membranes.—The most notable example of carcinoma following chronic inflammation is probably that observed in the gall-bladder from cholelithiasis. The disease forms 5

or 6 per cent. of all carcinomas (Kaufmann), and is four or five times as frequent in women as in men. Gallstones were present in 69 per cent. of Musser's 100 cases; 70 per cent. in Futterer's; 85 per cent. in Zenker's, 91 per cent. in Courvoisier's, 95 per cent. in Siegert's, and 100 per cent. in Janowski's. The proportion of cases of cholelithiasis which develop cancer is placed by Rolleston at 4 per cent., by Slade at 18 per cent. Slade found microscopical cancer in ten of seventeen cases, 59 per cent. Yet Candler found only two gross cancers in 315 cases of gallstones among the insane. Since cancer rarely produces gallstones, the calculi cannot be regarded as the result of the tumor. The tumor arises early in the course of lithiasis and takes a papillary or glandular character, or late, and then takes the form of infiltrating or fibrocarcinoma.

Aschoff and Pels-Leusden believe that irritation by cholesterin granules in the tubules of Luschka's gland (branching off from the vesical mucosa) is a frequent source of gall-bladder cancer. Menetrier points out that the proliferating glands especially in the polypoid outgrowths apparently at the stage of simple adenoma are capable of invading muscularis and lymphatics.

A well-definied form of hepatic carcinoma arises from chronic irritation and stasis of the larger bile ducts.

There appears to be something in the chemical or mechanical nature of the irritation by cholesterin which is peculiarly effective in producing atypical proliferation of epithelium.

In the uterus chronic catarrhal endocervicitis precedes cancer in the great majority of cases (34 of 48, Polese). The cervical erosion is the most definite established lesion known to precede cervical

carcinoma. Next comes leucoplakia of portio and canal which is often combined with erosion. Beckmann carefully watched the development of carcinoma in an erosion which he treated for five years. Early stages of the development of carcinoma from such lesions are described by Waldeyer, Ruge and Veit, Cullen, Scharenstein, Sitzenfrey, and others. I have studied three quite perfect examples of precancerous polyps of eroded cervices showing metaplastic overgrowth and just beginning invasion of the stroma by adenocarcinoma. In one case a squamous cell carcinoma is beginning lower down in the cervix.

For corpus carcinoma the chief definite etiological factor is the association with myoma, 10 per cent. of which, according to Olshausen, give rise to carcinoma in the overlying and chronically irritated mucosa. Local hyperemia and chronic endometritis are constant accompaniments of myoma, and ulceration is frequent. I find that many stages of inflammatory overgrowth up to adenoma and adenocarcinoma may be followed by routine study of these cases. Leucoplakia of the endometrium is chiefly associated with the corpus carcinoma of elderly subjects which takes the form of acanthoma or adenoacanthoma (Benckizer). Yet many cases of leucoplakia uteri run a prolonged benign course (Zeller).

Although Gebhard claimed that malignant adenoma commonly arises from the hypertrophied glands of chronic endometritis this simple explanation has not been fully verified. Heurlin in 44 cases failed to find any transitional stage or any glandular endometritis in the outlying mucosa. Yet 29 of his 44 cancers were diffuse. It seems probable that when corpus carcinoma develops on glandular

hyperplasia it first passes through an adenomatous stage. In routine material I find such transition stages rather frequent, but clinical observations in this field are still inadequate to prove the close dependence of corpus adenocarcinoma on inflammatory processes. I have only once observed a very marked grade of glandular overgrowth recur as adenocarcinoma in curettings received after an interval of two years.

In the urinary bladder the great majority of cancers can be traced to previous or coexistent cystitis. The multiplicity of vesical papillomas indicates the existence of widespread lesions in the mucosa from which tumors may develop. Stoerk has traced a definite relation between cystitis cystica and multiple papilloma, and he and Cahen report cases of the former developing into carcinoma.

Specific vesical irritants as observed in anilin workers are especially effective in producing vesical carcinoma, chiefly at the ureteral orifices (Rehn, Leichtenstern). A high proportion (50 per cent.) of these tumors are malignant (Seyberth).

In Bilharzia disease there is a series of changes, including venous and lymph stasis, catarrhal cystitis, irritation from ova, and lithiasis, which have been shown by Goebel and others to lead in many instances to carcinoma.

In epithelioma of the penis, which forms from 1 to 3 per cent. of all carcinomas in males, phimosis and syphilis are very common antecedents. Demarquay found phimosis in 85 per cent. of his cases and Kaufmann saw balanitis with warty vegetations in 29 of 33 cases. Hebrews are practically immune. Thompson describes as a precancerous condition a catarrhal balanitis with desquamation followed by hypertrophy of epithelium and subepithe-

lial connective tissue. Schuchardt calls this condition psoriasis preputialis.

Buccal and lingual cancer stands second according to Jessett in order of frequency, third according to Jacobson, and fourth in Winiwarter's statistics and shows a mortality of 75 per cent. to 90 per cent. (Warren, Meller). This disease is preeminently due to inflammation and local irritation. The combination of syphilis, tobacco, and bad teeth is present in a large group of cases and one of these factors almost always exists. While Meller found signs of lues in only 7 of 207 cases, Fournier regarded 155 of 184 cases as luetic. The syphilitic process usually takes the form of leucoplakia, of which 30 per cent., according to Fournier (15 per cent. Darier), develop carcinoma. In these cases there is hypertrophy and hyperplasia of epithelium, hyperkeratosis, lymphocytic infiltration of submucosa, papillomatous overgrowth, and finally destructive invasion of the deep tissues. All these processes require time. According to Darier the complication of an ulcer or fissure is usually necessary before leucoplakia becomes cancer. Other syphilitic lesions observed to precede cancer are wart-like hypertrophies, fissures, gummas, and chronic atrophic glossitis (Jacobson). The syphilitic process may completely heal while cancer develops later in the scar under a smooth epidermis. As long ago as 1872 these various buccal lesions were designated by Hutchinson as precancerous.

Physiological involution of organs and the complicating mechanical, nutritional, and inflammatory processes, precede the development of cancer chiefly in the breast and prostate. Definite neoplastic tendencies in the "maladie de Reclus" were first pointed out by Brissaud and by Schimmelbusch and con-

firmed by many later writers. It is especially in that form of chronic mastitis in which cysts and epithelial proliferation are prominent from the first that cancer is observed to develop, but no anatomical form of the disease is free from the dangers of malignant change. Bloodgood prefers to regard most of the cases as pure senile involution. Regarding the relation to cancer, opinions are as usual at variance. It is quite true that certain cases pursue a benign course for many years. Hence Delbet and many surgeons consider the disease as essentially benign although incurable. Against this conclusion stands a large body of clinical evidence which has been steadily increasing in recent years. Billroth stated that small cysts are very often seen about mammary cancers. Tietze described five such cases in detail and Verga reports a similar series. Tietze concluded that 10 per cent. of cases of chronic cystic mastitis develop cancer and Speese in 295 cases found 15 per cent. showing carcinomatous areas. Greenough and Hartwell found three cases of cancer in 30 of chronic mastitis, and Warren reported 15 in 150 cases (13 per cent). When one includes various suspicious areas histologically resembling cancer but not admitting of positive interpretation the proportion of precancerous conditions in chronic mastitis becomes much higher. In my material for the analysis of which I am indebted to Dr. B. J. Lee, it reaches at least 25 per cent. In cases of mammary carcinoma interstitial mastitis is practically constant but may be secondary.

Brodie, Brissaud, Reclus, Sourice, Schimmelbusch, Saar, and many others have declared that the step from chronic mastitis to carcinoma is short and often traversed.

In the prostate the chief condition predisposing to cancer is chronic prostatitis, usually with hypertrophy. The principal age of incidence of both conditions is the seventh decade when 68 per cent. of the carcinomas occur. Notable examples of the transformation of simple hypertrophy into carcinoma are recorded by many observers (Wolff, Kaufmann, Albarran, Halle, Greene and Brooks, Young). The evidence usually consists of a long history of hypertrophy in cases presenting carcinoma. At least 10 per cent. of prostatic cancers give such history. Among 500 cases of prostatic obstruction Young found 20 per cent. cancerous, and he states that about 15 per cent. of enlarged prostates after 50 years are cancerous. The proportion is placed by Albarran at 14 per cent.; by Walker at 16.5 per cent.; Wilson and McGrath, 15.5 per cent.; Freyer, 13.3 per cent. In most of these cases the process was interrupted by operation and its capacity to develop cancer was not revealed. Wolff saw carcinoma develop after incomplete extirpation of an enlarged prostate. Whatever the nature of prostatic hypertrophy may be it is clear that the influence of physiological involution is usually concerned.

Regenerative hypertrophy may be regarded as the predominant underlying influence in the development of certain forms of cancer, of which the best examples are seen in the thyroid gland and liver. In fish the functional overactivity and hypertrophy of the thyroid observed in crowded ponds where the animals are fed on protein diet leads in a small proportion of cases to maligant overgrowth, which is a specific form of cancer. This condition has been reproduced under experimental conditions and fully traced by Gaylord. There is reason to be-

lieve that the thyroid cancer of young girls and possibly at later ages sometimes arises under parallel conditions. Few cases of thyroid carcinoma develop in subjects with entirely normal thyroid history, but many follow goiter, interstitial thyroiditis and Graves' disease.

In the liver an important group of primary carcinomas or hepatomas represents malignant overgrowth of regenerating lobules following injury with degeneration or cirrhosis (Hanot, Gilbert, Menetrier.) Probably many tumors of the kidney, adrenal and hypophysis are of the same nature.

The transformation of benign into malignant tumors is a very frequent source of cancer, universally recognized, occurring in nearly all organs, and of prime significance in considering the origin of cancer. Many of these benign tumors are clearly of inflammatory origin, and the organs may present every gradation between simple inflammatory overgrowth, adenoma, and carcinoma. The most striking example occurs in the gastrointestinal tract and especially in colitis polyposa. This disease affects both old and young subjects and exhibits an hereditary, individual, and local predisposition. In the early stages the epithelium presents a peculiar and excessive reaction to some irritant, and catarrhal symptoms are marked. Then follow localized polypoid outgrowths, multiple adenomas, and carcinoma. According to Quenu and Landel about half the cases develop carcinoma. Hauser found this combination the rule, and of 50 cases analyzed by Doering 31 of 37 fatalities were from carcinoma.

The transformation of single benign gastric and intestinal polyps into carcinoma has been described in detail in a series of communications by Menetrier. According to this observer gastric ulcer

leads to cancer through the preliminary development of polypoid adenomas on the edge of ulcer.

The foregoing brief series shows how very numerous are the pathological processes, which in a high proportion of cases are known to be followed by cancer. The list might be still further extended. These clinical observations form the chief basis for the classification of many cancers in the so-called "irritation group." The data cited are almost entirely clinical. The events in sequence ending in cancer are merely observed to follow one another, but this sequence does not prove that there is any essential relation between the processes. It is, therefore, questionable if the term precancerous disease is legitimately chosen. In fact, many have claimed that when mammary cancer follows chronic mastitis, one disease has been grafted upon an entirely different disease. It has been affirmed that a pathological condition is either cancer or not cancer.

To combat this highly mechanical notion of biological processes it is necessary to show that there is an essential connection between the preliminary disease and the resulting cancer and this can be done only by close microscopical study of precancerous conditions. This study has fully demonstrated that an extensive series of structural changes takes place in the precancerous diseases leading up to the fully developed cancer, the one passing by insensible gradations into the other. It is not true that a pathological condition must be either cancer or not cancer. It may be neither the one nor the other. It may be in the process of becoming cancer. This process takes time, sometimes years, and may be accompanied by very striking histological changes which may be difficult to classify, since they lie between inflammatory and

neoplastic hyperplasia. To this series of intermediate stages of hyperplasia revealed by the microscope in diseases clinically observed to pass into carcinoma the term "precancerous lesion" may be applied.

I believe, however, that only when the preliminary lesion shows a definite advance into the field of genuine cancerous change can the term precancerous be properly applied. Otherwise almost every form of chronic productive inflammation may be regarded as precancerous since in some instances the particular condition clinically passes into carcinoma. Yet not every case of chronic mastitis shows suspicious changes suggesting carcinoma. When any case does exhibit the suspicious structures then and only then can it be regarded as precancerous.

I believe, further, that a distinction should be made between precancerous lesions and miniature carcinomas. All the local signs of malignancy may be exhibited in comparatively minute foci of cells, and may be observed in chronic mastitis, and while the circumscribed lesion may not reveal its potentialities to the clinical observer the microscope detects the malignant capacities with complete certainty. Only in size do such miniature carcinomas differ from the established disease. Such lesions cannot properly be called precancerous since they exhibit the essential features of fully developed carcinoma, only on a small scale.

The data which support the principles here stated are derived from the microscopical study of cancer and precancerous lesions and their elucidation requires an analysis of the essential features of the carcinomatous process. Some features of this analysis may here be considered.

Histological Criteria of Carcinoma.—It must be noted that the histological criteria of carcinoma differ greatly in different organs and that only by clinical observation have we been able to determine and predict the momentum and the probable course of different malignant tumors. The significance of the various changes belonging to the cancer process must be learned for each organ. Moreover, cancer of the same organ exhibits in widely different degrees the histological features of carcinoma. Thus some malignant cancers of the thyroid differ so little from the structure of the normal gland that Cohnheim called them benign metastasizing struma, and Riedel, Oderfeld, and Steinhaus erroneously concluded that the normal alveoli of the gland might pass into the blood stream and produce distant tumors. Here the metastasizing property was developed far in excess of other carcinomatous features. On the other hand, extreme grades of somewhat atypical cellular hyperplasia with giant cells and ill-formed alveoli are observed in the wholly benign goiter of Graves' disease.

In the stomach a comparatively orderly adenomatous hyperplasia is usually malignant and may be found in distant metastases, while in the rectum a similar degree of glandular overgrowth constitutes a harmless polyp. It is extremely difficult to establish any universal criteria of the cancer process and I find that some who have attempted to do so encounter many difficulties. Thus Borst applying a somewhat rigid conception of carcinoma hesitates to accept many very malignant hepatomas as true cancers because they fail to show the local aggressive properties of many carcinomas and are mechanically forced to grow into the hepatic vessels. These few illustrations among many that might be

cited, suffice to show that there are many morphological details in cancer processes, and that all are not equally developed in every genuine cancer.

Probably the best definition of the cancer process is "atypical and destructive proliferation of epithelium." Yet many well known forms of cancer fail to meet these requirements and it becomes necessary to analyze the process in much greater detail in order to admit many malignant diseases into the cancer category. This analysis requires the recognition of at least the following criteria of a malignant process: (1) Cellular overgrowth passing beyond that observed in other processes affecting the same tissue; (2) atypical qualities of the cells, metaplasia, anaplasia; (3) loss of polarity; (4) heterotopia; (5) desmoplastic properties; (6) local invasive and destructive properties; (7) metastases. It will be observed that in each of these characters variations in degree are conceivable and, as a matter of fact, they are commonly observed to occur.

Any one of the above attributes of cancer may be almost exclusively represented in the disease. Excessive cellular overgrowth difficult to distinguish from a physiological type chiefly characterizes some thyroid cancers. Atypical qualities of the cells are the only safe criteria of early malignant papilloma of the larynx. Loss of polarity is the chief feature of certain large alveolar mammary carcinomas confined within ducts. A pronounced fibrosis about slightly altered prostatic alveoli was the most definite feature of a fatal case of prostatic cancer which I have recently studied. Local invasive and destructive properties first reveal the beginnings of some lymphosarcomas. Distant metastases are the chief evidence of malignancy in

the peculiar thyroid cancers previously mentioned. Hence the diagnosis of cancer becomes a matter of judgment as to the significance of any one or all of the above features that may be combined in any one cancer. The disease is not always one and the same thing. On the contrary, it is a progressive process which has small and variable beginnings and unfolds more and more of its features as it advances to a fatal issue. It is from this point of view that the significance of precancerous lesions must be regarded. From this standpoint such lesions may be defined as pathological processes which show some, but not all, of the structural features which characterize fully established cancers of the affected organ.

To what extent, then, are the criteria of cancer presented in the various conditions designated as precancerous diseases? Time fails for a full discussion of the many precancerous lesions in the several organs, but a few illustrative cases may be considered, of which the breast is perhaps the most fertile field.

The gradual passage by many transitional stages of the nutritional and inflammatory changes of chronic mastitis into cancer has been traced by many observers and in such detail as to leave no reasonable doubt that these authors have been describing stages of one and the same process. It may be fully admitted that these stages in order to form an unbroken chain must usually be taken from a series of cases, no one breast furnishing them all. Constructed in this way the fabric of proof is without a break.

Prominent among the early changes is the development of small cysts of lacteal, interlobular, or intralobular ducts filled with puriform, fatty, or

blood-stained material. Into these cysts project low papillæ of proliferating epithelium which gradually enlarge, exhibiting a grade of overgrowth which passes the inflammatory limit. No other neoplastic feature may be present and we speak of the lesion as papillary adenoma. Since the papillary outgrowths may recede after evacuation of the cyst many of these lesions in the breast and other organs must be regarded as inflammatory and not adenomatous. Yet in others the growth becomes progressive. Other cysts show marked increase in the size of the cells and nuclear hyperchromatism. Loss of polarity appears in disordered multiple layers of cells but there is yet no heterotopia, which is added when stroma or capsules are invaded by atypical cells completely displaced from their normal attachments. Or, loss of polarity may be prominent from the first when the cyst is lined by multiple layers of atypical cells. Thus may be traced the development of the series of cyst adenomas and carcinomas, many of which develop in chronic mastitis. In other cases cysts are not prominent, but an interstitial productive process affects the lobule. The acini show from the first a notable tendency toward overnutrition and overgrowth, interpreted by Brissaud as a neoplastic process. The acini assume the characters of ducts as in the fetal breast. The cells multiply producing lateral sacculi and many new regular alveoli as exclusively seen in one form of adenoma; or more often the cells multiply rapidly, become atypical, exhibit loss of polarity, heterotopia, and soon local invasive properties. By these stages many of the circumscribed hard fibrocarcinomas develop in chronic interstitial mastitis.

Periductal fibrosis affecting one or more isolated portions of the breast may lead to extensive pro-

liferation of lining cells which in various phases exhibit simple overgrowth, later atypical cell characters, loss of polarity, diffuse growth within a limited segment, then heterotopia and invasive properties.

Both ducts and acini participate in the abnormal proliferative changes, but in most cases the acini assume the characters of ducts and the distinctions between the two structures are obliterated. The aberrant sweat glands described by Moullin, Dryfuss, and Krompecher are often involved, producing cysts lined by strongly acidophile cells. The tumors derived from these cysts contain large granular pavement-like cells. In other cases the proliferation of acini is the predominant feature.

In the single case of chronic mastitis the suspicious areas are usually multiple and often numerous. They are best detected by examination of the breast in the fresh condition when they appear as unusually hard shotty nodules, or as small cysts with softened walls, or as cysts with opaque cellular contents. Of ten such nodules chosen from one breast in a case of Gibson's I found three groups of distended ducts diffusely filled with atypical cells and one miliary cystadenoma. The whole breast may be uniformly affected or the proliferation may be most advanced and atypical in one segment. As a rule the suspicious areas are more abundant in the more advanced cases. In some very small and very atrophic breasts I have found distinct precancerous lesions or miniature carcinomas. These cases correspond to those in which a very small breast is everywhere transformed into carcinoma. Thus several different gross forms of carcinoma seem traceable to the types of precancerous changes observed in chronic mastitis.

The proportion of cases of chronic mastitis which

may be classed as showing precancerous lesions depends largely on the standards employed in interpreting abnormal proliferation. It should be emphasized that considerable proliferation and desquamation of epithelium is seen in nearly all cases of chronic mastitis and senile involution and unless quite specific changes are demanded before employing the term precancerous, one must consider all cases of chronic mastitis as dangerous. Widely distended ducts diffusely filled with atypical cells, pronounced multiplication of alveoli with obliteration of acinar outlines, and miliary adenomas with areas of diffusely growing large atypical cells, would seem to require recognition as tending distinctly toward malignant growth. Some of these lesions closely approach miniature carcinomas, but are restrained by the thickened walls of ducts or other fibrous barriers. The comparative frequency of such lesions in the breast at many ages suggests that when carcinoma is observed to follow trauma it is through the liberation of cells growing under confinement in the cysts and proliferating ducts of chronic mastitis. Likewise when carcinoma develops after imperfect extirpation of fibroadenoma the trauma may liberate and accelerate the growth of the suspicious areas often seen in or about benign tumors.

Notwithstanding the close resemblance of precancerous lesions in the breast to carcinoma the actual passage of the one into the other is not to be accepted without searching criticism.

It may be that the suspicious lesions never pass beyond the stage at which they are observed. Only a minority of cases of chronic mastitis developed carcinoma, while atypical epithelial proliferation is rather common. Moreover, not a few cases of chronic mastitis progress for years without malig-

nant transformation. It is necessary to admit that the mere discovery of precancerous lesions does not assure in every instance that the process was bound to become carcinoma.

Nevertheless that some of the lesions do progress to carcinoma is a conclusion that cannot well be doubted. The series of transitional lesions in a series of cases or even in the same case is complete. The law of the momentum of pathological processes justifies the deduction that when atypical overgrowth begins and the conditions inciting it persist, the proliferation tends to become more and more atypical and active. In accordance with this law is the observed fact that a high proportion of cases of recognized mastitis develop carcinoma. Even more convincing is the still higher proportion of cases of carcinoma found to occur in breasts which are the seat of unrecognized chronic productive changes.

The most serious argument against the theory of precancerous lesions is the fact that many carcinomas are not proven to be preceded by such changes. Some of the very earliest cancers observed in the breast have been too far advanced to permit tracing them to their cells of origin. Ribbert states that no one has ever seen the beginnings of mammary cancer. Moreover, when cancer does develop in chronic mastitis it very soon overgrows and obliterates the original lesions. There is often a perceptible gap between the atypical proliferation and the smallest established carcinomas. Hence comes the impression that when carcinoma is grafted on mastitis a wholly new disease is added. I have seen rather numerous illustrations of the principle that when carcinoma develops in precancerous lesions the change may be sudden and the progress of the new lesion rapid. It appears that the precancerous

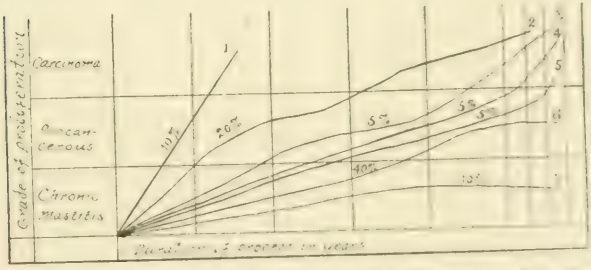
lesions that become cancerous may reach the malignant form early in the course of the precancerous disease or without a period of slowly developing malignant properties. Hence there is no uniform relation between the progress of chronic mastitis and the resulting cancer and some chronic cases becoming established on a benign basis maintain that character throughout. Perhaps equally important as an explanation of the sudden unfolding of malignant characters is the release from mechanical pressure which proliferating cells experience when they once break the barrier of basement membrane.

The above conceptions may be elucidated by the diagram.

None of these considerations seems to me to seriously affect the interpretation of precancerous lesions as a preliminary stage of mammary carcinoma. Morphological and clinical studies as well as theoretical data accord in supporting this interpretation. There are other ways in which mammary cancers develop, but this is one and probably the most frequent. Clinical observations bearing on precancerous conditions in other organs have been presented at sufficient length to show that the breast is not peculiar in this relation but that the development of cancer from precancerous lesions is a principle of wide application. In each organ, however, the details of the changes from harmless to malignant epithelial proliferation are peculiar and require study and interpretation by themselves.

Important practical results follow acceptance of the doctrine of precancerous lesions. If inoperable advanced cancer is incurable, and localized cancer eradicable, the disease is preventable by dealing with its preliminary stages. Precancerous lesions are not cancer. Practically they differ enormously

from the established disease. They can usually be removed by trivial or safe operations, and they are sometimes amenable to less violent treatment. The therapeutic problem of dealing with cancer, as it were, before it exists, has made far greater progress than the doctrine of precancerous lesions. Gastric ulcers, lingual warts, fissures, and placques, eroded cervices, pigmented moles, and benign tumors, are everywhere excised, with the conviction that a malignant tumor may thereby be prevented, but the relation between the benign and the malignant process is still under discussion and often frankly doubted.



1. Cases passing rapidly through the intermediate period of atypical or precancerous changes. 2. Prolonged period of precancerous changes with slowly progressing carcinoma 3, 4, and 5. Rapid development of carcinoma or well established mastitis. 6. Late appearance of precancerous changes 7. Prolonged chronic mastitis remaining benign.

In the breast the therapeutic problem is unusually complex.

Although the majority of mammary cancers arise in previously altered breasts the preliminary changes often fail to attract the patient's attention. Yet there is a large group of cases which present lumps, nodules, cysts, and thickenings requiring

diagnosis and treatment. While the decision in these cases belongs to the surgeon, the observations of the pathologist have an important bearing in this field. In general I think it may be said that gross and microscopical study tends to emphasize the dangers of benign tumors and chronic inflammations of the breast. The local removal of fibroadenomas, especially in young women, can be fully justified by the intact conditions generally found in the remaining portion of the organ. In older women I find these tumors more often multiple and associated with chronic mastitis, and in some cases carcinoma has developed after excision of wholly benign fibroadenoma. Its sources in these cases are not clear. Simple cysts in otherwise normal breasts often spontaneously regress, but I find no adequate clinical study of the subsequent course of these cases.

A group of cases presents nodular thickening of one segment only, of the breast, partial chronic mastitis, and there are numerous records of the successful removal of the diseased segments. There are also numerous cases in which carcinoma has developed in the remnants of such breasts, or in the other breast. I have observed three fatal cases with this history. Hence partial extirpation for chronic mastitis must be regarded as only partial insurance of the life of the patient. The best procedure in these cases may be determined by the possibility of closely following the condition after operation. The practise of excising small portions of the breast and submitting them to hasty study by frozen sections, while not without value, is fraught with danger. The pathologist can pass only on the material examined, not on the whole breast. If any operation is performed on an indurated or

cystic breast the whole affected area or the whole breast should be removed. The subsequent procedure can then be determined almost invariably by minute gross examination.

The treatment of established chronic mastitis when the condition can be recognized as such by clinical examination has not yet become uniform. Delbet appears to represent the practice of French surgeons in pronouncing the disease benign and not requiring radical treatment. He recommends that a strict watch be placed over these patients and believes that a malignant transformation can be detected in its early stages when operation is almost surely curative. Baumgartner only partially dissents from this view. Yet French observers first pointed out the dangerous tendencies of the disease and Cornil emphasizes them in his recent work. The treatment recommended by Delbet—compresses, incisions, and iodine injections—is ineffective, and the condition tends to become an increasing burden to the patient. The late stages even of the benign cases described by Billroth are suspiciously like scirrhus carcinoma.

In America I judge that surgeons are more and more inclined to believe, if not fully agreed, that diffuse chronic mastitis demands radical treatment. In 1905 Warren devised for this purpose the operation of plastic resection of the breast, leaving skin and nipple, and this mode of dealing with the disease, with appropriate restrictions, was fully endorsed by Gibson, not only for pronounced chronic mastitis, but for many benign tumors which are often associated with mastitis. While some unnecessary surgery may thus be done I think the study of precancerous lesions in this organ fully justifies the radical procedure.

Presarcomatous Lesions.—The occurrence of atypical productive inflammatory changes leading to sarcoma is not as widely recognized as are precancerous lesions, but is fully in accordance with established views regarding the nature of sarcoma. In fact, many sarcomas show such marked resemblance to inflammatory diseases that pathologists have long been prepared to accept, in a certain sense, the inflammatory or even the parasitic origin of certain sarcomas. The lymphosarcomas especially stand to-day in this position, and Borst among others anticipates the ultimate identification of this group of neoplasms with the infectious granulomas.

Relation of Tuberculosis and Syphilis to Lymphosarcoma.—That tuberculous lymphadenitis may pass rapidly or after several recurrences into lymphosarcoma has been long indicated by clinical observations. Ricker, in a case terminating after fourteen years, found frank tuberculosis associated with lymphosarcoma of neck, lung, adrenals, and spinal cord. Muller saw general sarcomatosis from a primary tumor of the breast, miliary tubercles being nearly coextensive with widespread sarcomatous tumors. Brandt described a case of apical tuberculosis with sarcomatous invasion of ileocecal region, pharynx, parotid, adrenal, and ovary. Inoculation of this pure sarcomatous tissue gave hyperplasia of intestinal follicles in guinea pigs, but inoculation of the tissues of these follicles gave frank tuberculosis in a second series of guinea pigs. Tuberculous intestinal ulcers associated with sarcoma of mesenteric and retroperitoneal nodes is described by Nothnagel, Freudweiler, and Munk. Since the discovery of tubercle bacilli in pseudoleucemic tissues by Weigert; in pure lymphoid hyperplasia by Brentano and Tangl; in generalized

lymphomas of nodes, spleen, liver, and serous membranes by Sabrazes, only a slight difference has appeared to separate certain lymphosarcomas from the immediate or distant presence of tubercle bacilli or their toxins. Probably other infectious granulomas may also hold a similar relation, and various miscellaneous and undetermined infectious processes have apparently given rise to the disease.

The transformation of Hodgkin's granuloma into sarcoma has now been fully traced by several observers (Yamasaki, Karsner, Welch). In Welch's case the first nodes showed atypical tuberculosis or Hodgkin's granuloma, but when the patient died two years later there were sarcomatous tumors of lung, liver, and spinal dura. The transformation usually occurs in the late stages of the granuloma, but in a recent study I pointed out that tumors identical in structure with those following Hodgkin's disease are observed in sarcomas of recent origin and limited to the axilla.

In this study was reported a case of cervical tumors, probably tuberculosis, recurring four times in six years and terminating with the picture of endothelioma of lymph nodes. In this study it was also shown that an infectious granuloma, probably tuberculosis, may cause proliferation of the reticulum cells producing the large cell lymphosarcomas, or of the lining endothelium of the lymph sinuses producing a series of endotheliomas of lymph nodes.

The difficulty of distinguishing certain late syphilitic lesions in muscle and bone, socalled "sarcoma syphiliticum," is mentioned by many authors, and especially by v. Hansemann. The recurrence after operation, multiple appearance, and resistance to treatment also complicate the clinical diagnosis

While the active stages of these lesions strongly resemble spindle or round cell sarcoma, they tend toward necrosis and cicatrization, rather than to progressive growth. That true sarcoma may develop from such lesions seems quite possible, but satisfactory proof of this transformation is lacking.

In the healing of fractures and of wounds of the periosteum the histological picture usually shows very marked overgrowth of regenerating capillaries, bone, giant, and connective tissue cells, and resembles the structure of sarcoma. I have observed chronic processes of this type in which it was impossible to determine whether one was dealing with inflammatory or regenerative overgrowth or early sarcoma.

Traumatic myositis ossificans appears to occupy a position intermediate between inflammatory and neoplastic processes (Berndt). The clinical history of established cases is that of a self-limiting productive inflammation, but the histology of the early lesions may be difficult to distinguish from bone sarcoma. It appears quite possible that the division of cases is accidental, some of the early lesions progressing as myositis, others as traumatic sarcoma. In a case studied in this laboratory and published by Coley the two conditions were combined. Kolisko finds that osteitis fibrosa may be followed by progressive changes leading to giant cell sarcoma of moderate malignancy.

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ANATOMICAL STUDY OF A VERY EARLY TUBAL PREGNANCY, WITH SPECIAL REFERENCE TO THE QUESTION OF DECIDUAL REACTION IN ECTOPIC GESTATION.*

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(With thirty-one illustrations.)

Among the numerous studies published to date on the pathology of ectopic pregnancy, it will be sufficient to mention those of A. Martin, Abel, Kossman, and Werth. The most extensive and complete anatomical researches on the different questions involved in the ectopic imbedding of the human ovum are still embodied in the classical works of Webster and Couvelaire.

This last author has established the principles for subsequent studies requiring that researches be conducted on specimens of ectopics in evolution, and not on hematosalpinx of more or less old standing, or in others in which the mutual relations of ovum and tubal wall are completely destroyed by internal or external rupture of the embryonic sac.

Upon the basis of a study of more than fifty specimens of ectopic, with special attention devoted to implantation and formation of decidua in the tube, I feel safe in stating in advance that the earlier the age of the ovum, the more reliable the data, as compared with those offered by early uterine ova.

Of the long series of cases which I investigated in the Pathological

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Department of Cornell University, I found one worthy of careful and complete study, on account of its early age and the relative integrity of the component structures.

The rare occurrence of early, noninterrupted ectopic gestation is a fact fully appreciated by every one, who clinically at least, is well acquainted with the subject, and in fact, the most accurate study of the literature shows that if the early uterine ova are few, the early tubal are extremely rare.

In the first place the most common date for the interruption of the ectopic is generally admitted to be from the fifth to the seventh week. For the isthmic part, however, the investigations of Couvelaier have established that owing to the anatomy of this part of the tube the termination is more precocious.

In the second place, unless the accident is recognized with the initial attack, and the operation immediately performed, the chances of finding the relations of the structures still maintained are very few.

The only possibilities left for such occurrence are occasional findings of tubal pregnancies during operations performed for other indications, or *postmortem*.

In my case two fortunate circumstances contributed to the finding of a young ovum relatively well preserved implanted in the tube: (*a*) Seat of imbedding, isthmic part of left tube; (*b*) almost immediate operation after the first symptoms.

The more advanced cases, even if found in perfect state of evolution, could never give such evidence of the mutual relation between ovum and tube as the early ones, especially as far as the decidua is concerned.

Even excluding the usual delay interposed between the accident and the time of operation, the most possible factors of the altered relations and disappearance of the structures before the time of the accident, are the relatively poor trophic changes of tubal ova and the common intertuboovular extravasation of blood.

As a natural result of these causes and the difficult accommodation of the tubal wall to the irregularity in the increasing intratubal pressure, the tissues become necrotic, and the decidua is the most affected.

I will give now a summary of the case, which in the details substantiates the anatomical findings.

Mrs. C. M., thirty-one years old, Italian. Menstruated at fourteen years. Always regular—four to five days type.

No evident account of any infection previous or after marriage. Married four years before present illness. One child three years old. Normal labor and puerperium. Sterile since.

Periods always regular up to the fifteenth of January, 1912, which period lasted five days. No menstruation in February. On the seventeenth of February in the afternoon the patient was seized by severe abdominal and pelvic pain, with fainting spells, slight show of dark colored blood.

When I saw the patient she was practically in collapse and profoundly anemic.

Abdomen distended and tender. No masses could be felt in the pelvis. Uterus moderately enlarged and soft, external os impervious to the examining finger.

Patient had not seen any passage of membranes. Having made a positive diagnosis of ectopic, I operated upon the woman three hours after the beginning of symptoms.

FIG. 1.—Left tube and ovary. The tube was removed deeply into the horn of the uterus. The ovum occupies the isthmic section of the tube. Site of the rupture evident on the upper aspect of the tube.

At the operation a moderate quantity of free blood was found in the abdominal cavity. No clots. Uterus and adnexa were brought to view, and a careful inspection of the left adnexa revealed the presence of a continuous moderate oozing of blood from a slightly noticeable punctiform rupture in the upper side of the isthmic portion of the tube, quite close to the uterine end.

Left adnexa were removed. The patient made an uneventful recovery, and has had one child since.

Gross Specimen.—The specimen as represented in Fig. 1, minus a section which had been previously taken for examination, shows the

left tube and the ovary attached. The ovary, as it appears, is the seat of the corpus luteum of pregnancy.

The tube in the isthmus, quite close to the interstitial part, shows an almond-shaped enlargement, the longitudinal diameter of which is 15 mm. and the transverse at the center about 11 mm.

Almost in the middle of the upper side of the enlargement a punctiform rupture is seen.

Microscopical Findings.—I have made more than 300 sections of the entire ovular sac, and distal and proximal portions of the tube

FIG. 2, x 17.—Section taken at the site of the rupture. Blastocyst in the center; triangular in shape, surrounded by blood, trophoblastic masses and villi. Most of the villi sparse on the periphery close to the inner surface of the tubal wall.

outside of the sac. I failed to secure serial sections of the specimen, and probably on that account missed the embryonic area.

Blastocyst.—In many of the sections, specially the central ones, I have been able to trace a well preserved blastocyst. This is represented by a central cavity completely surrounded by a chorionic layer (Figs. 2, 3, 4, 5).

The dimensions of the blastocyst cannot be very easily determined. In some of the microscopical sections, looking with the naked eye and approximately measuring the empty space in the center, repre-

FIG. 3, x 16.—Blastocyst of identical shape of No. 2. Different arrangement of trophoblast at the base.

FIG. 4, x 16.—Blastocyst of quadrilateral shape. Cavity of blastocyst smaller. Villi attached in many points of the chorionic wall.

senting the cavity of the chorionic vesicle, the longest diameter varies from 3 to 4 mm.

This figure is better appreciated and determined from a magnification of the entire section which is sixteen to seventeen times the natural size. We must allow much more for the actual size when we take in consideration the collapse of the chorionic vesicle and the shrinkage induced in the tissue from the fixation fluid.

The chorionic vesicle assumes different shapes as can be seen from the accompanying illustrations (Figs. 2, 3, 4, 5), crowded in the center and thrown more or less into folds by the blood extravasation.

FIG. 5, x 16.—Section taken at the proximal pole of the ovum. The blastocyst is surrounded almost entirely by blood and fibrin.

In any one of the sections, comprising the entire area, the details evidently show that the blastocyst has lost all the primitive connections and relative position of implantation.

The enormous amount of blood and fibrin surrounding the blastocyst, the very few villi closely connected with it, most of which are left spread out in the periphery attached to the maternal tissue, demonstrate the fact, that the specimen was secured when an internal abortion had occurred, possibly sometime previous to the operation.

The arrangement of the cells which compose the wall of the blasto-

cyst is well shown in Fig. 6. This wall is mainly made up of two layers of cells. In some parts, however, as many as six or seven layers of cells can be distinguished.

The differentiation of the cells is not possible everywhere, and a definite distinction is not easily detected except in a few spots of the wall of the chorionic vesicle, and only in some sections. Where such distinction is apparent, the cells of the internal layer are round or oval in shape, have very definite outline and show one or more, well-stained nuclei. The protoplasm stains very poorly, and that is

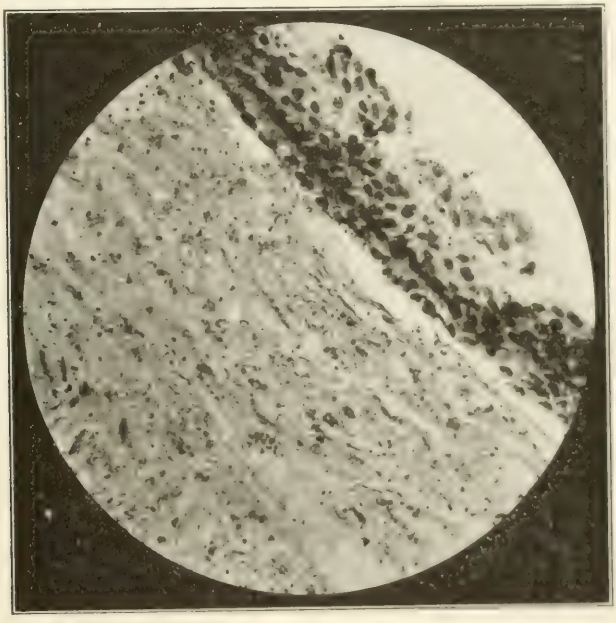

FIG. 6, x 450.—Chorionic wall of the blastocyst. Mesoblast.

probably the reason why it is not easy to differentiate them from each other. These are the cytotrophoblastic or Langhans cells.

The elements constituting the external layer are made up of irregular masses of protoplasm with no contour and small nuclei. The protoplasm is granular and assumes a deep eosin stain. The nuclei generally flat, are darkly stained with the hematoxilin.

These are the plasmoditrophoblast or syncitium. Where the layers are multiple, they are entirely made up of syncitial masses, except the innermost one.

No vacuoles or spaces are discernible in the plasmodial elements.

At intervals a circumscribed point of the wall shows from the inner aspect a depression which has the appearance of an elongated cavity, ready for the reception of the mesoblast, actually developing the villi.

The cavity of the blastocyst in the central sections appears to be partly filled with mesoderm, arranged in a more or less thick layer, paving the inner side of the chorionic lamella, and leaving in the center a more or less wide and irregular empty space as is shown by sections 2, 3, 4 and 5.

In the polar sections where the pressure had been greatest, and more perhaps on account of the tangential way the sections were cut, the mesoderm completely filled the cavity.

In the above-mentioned depressions of the lamella forming the wall, the mesoderm sends occasionally indentation processes to fill the space, constituting villi.

Anatomically it is made up of a mucoid intercellular substance, in which spindle-shaped cells are diffusely scattered.

As I have previously stated, in none of the available sections, have I been able to find any definite embryonic area. If that is due to the failure to secure serial sections or to a pathological condition of the ovum, or faulty development on account of poor trophic exchanges, it is impossible to say.

Chorionic Structures.—Going now into the details of the structures surrounding the chorionic vesicle, in spite of the altered relations of fetal and maternal structures, many important features are worthy of description, which correspond with the data acquired through the study of other early ova.

Even with the collapse of the blastocyst and the large quantity of blood present, we may approximately judge from the disposition of the villi, arranged all along the periphery of the implantation cavity, that they probably surrounded all the chorionic vesicle and connected it to the maternal structure.

Couvelaire has found the same condition in his youngest tubal ovum of four weeks. The chorion at this time is villous in all its extensions. Early tubal ova are identical to early uterine ova-placenta everywhere in the periphery.

Here and there as is represented in Figs. 7 and 9, villi are closely connected and even intimately attached to the chorionic wall of the blastocyst.

A careful study of the changes of the chorionic wall and the immediate epiblastic formation gives with the attached illustrations

a perfect idea of the successive evolutions in the formation of the villi.

The proliferative activity of the trophoblastic elements is greater in some parts of the chorionic wall than in others. Solid buds of trophoblast and completely formed villi are closely surrounding it. These solid buds of cells, as in Fig. 8, are almost entirely made up of vacuolated plasmodial masses, among which are occasionally seen distinct round or oval cells lightly staining, with one or more nuclei.

FIG. 7, x 75.—Villi attached to the blastocyst.

These buds, often isolated in many sections and independent from villi, are to be interpreted as remains of the primitive trophoblast or trophospongia of Hubrecht and generators of villi.

The mode of origin and formation of the villi, as described by Webster, is distinctly shown in my sections.

(A) In Fig. 7 we have one villus completely developed, made up of the epiblastic layer, including a mesoblastic but not entirely independent of its generator. The mesoblast is continuous with the mesoblast of the chorionic vesicle, as the trophoblastic layer is made of an extroflexion of the trophoblast of the blastocyst. In this

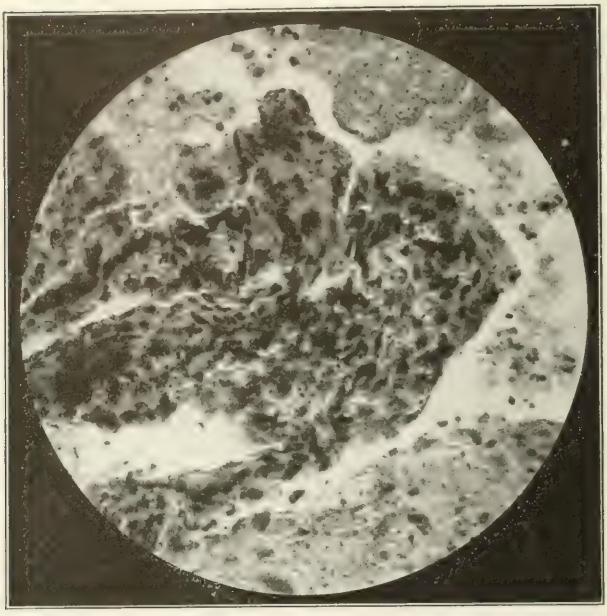

FIG. 8, x 450.—Plasmoditrophoblast masses, showing vacuolization.

FIG. 9, x 200.—Chorionic wall of the blastocyst in proliferation. Villi attached.

instance the villus was undoubtedly formed through simple extension of the mesoblast in a depression of the wall of the blastocyst.

(B) In the same illustration on the opposite side, masses of syncitium with few Langhans cells are extending outward as projections, close to highly developed villi. Vacuolization, peripherical expansion of the plasmodial trophoblast, and consecutive extension of mesoblast inside are evident in these masses. This genesis of the villi from the proliferation of the trophoblast first, and then extension of the mesoblast in the forming vacuoles, is the fact most gen-

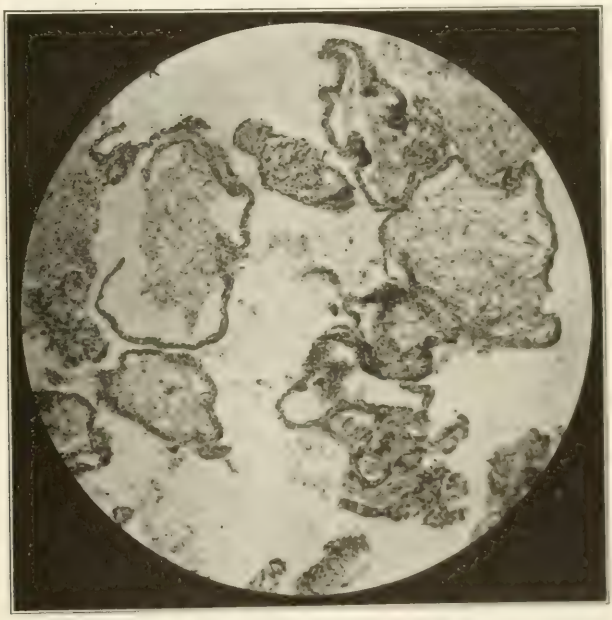

FIG. 10, x 60.—Masses and strands of plasmodium, villi in evolution.

erally accepted and is possible for all chorionic outgrowths, either connected with blastocyst or villi, or independent. Figs. 10, 11 and 12 clearly illustrate this point.

In the distinct network of plasmodial elements, mostly arranged in strands some of the spaces, within these strands, are incompletely filled with mesoblast, and some are completely filled with forming villi.

(C) Intermingled with the strands of syncytium is a solid group of Langhans cells (Fig. 12). Crowded out to the periphery and incompletely surrounding the central mass of cells, are plasmodial

elements, arranged as a continuous layer. Scarse mesoblast occupies the intercellular spaces.

From this finding, it seems probable that independent groups of cytotrophoblasts are apt to develop in complete villi, forming plasmodial elements from the cells crowded to the periphery, in which process of crowding out they find good support in the penetrating mesoblast.

Characters of the Villi.—The average size of the villi deduced from the magnifications of the microphotographs is from 1 to 2 mm. Most of them are round or club shaped. They are short, thick, few of them are long, but very few show any branches or attempt at

FIG. 11, x 175.—Plasmodial strands.

branching. The outer covering has the same histological appearance as the one described as forming the wall of the blastocyst. In some villi the distinction of the layers of the different trophoblastic cells is still clearer than in the wall of the blastocyst. The basal or internal layer, which in some villi is constituted by a few individual cells separated at various intervals, in many villi there is a true continuous and characteristic layer of cytotrophoblastic cells, with a comparatively distinct outline, showing one or two nuclei lightly

staining. The outermost covering of the villi is distinctly arranged in a single or double layer of irregularly shaped plasmodial masses with deeply staining nuclei, mostly flattened. Vacuoles are present, in the fine network of these plasmodial masses.

Buds or proliferating processes of the epiblastic covering of the villi, are frequently seen to grow from their surface, and consist almost exclusively of plasmoditrophoblast elements.

FIG. 12, x 330.—Plasmodium and cytotrophoblast masses, apparently evolving into a villus.

The mesoblast of the villi is exactly similar to the one found in the blastocyst. It is a loose network of homogeneous tissue of embryonic type, in which are lightly scattered round or branched cells.

Vacuoles are found in the mesoblastic meshes. No trace of capillaries.

All the structures described, strands of plasmoditrophoblast or cytotrophoblast, villi in formation, completely formed villi, are irregularly and diffusely scattered in the blood and organized fibrin surrounding the chorionic vesicle, detached as they were from fetal and maternal connection by the internal abortion.

The same structures unequally distributed, but still maintaining

their anatomical relations with the underlying decidua are seen in the periphery of the implantation cavity, but the complete details will be later described, after giving an account of the ovular sac.

Gestation Sac.—This, as I have previously stated, is constituted by the isthmic section of the tube quite close to the interstitial. The changes in the peritoneal, subperitoneal and muscular coats of the sac are few and of little importance. The serous coat, partly covered by flattened epithelium, is in some parts deprived of epithelium.

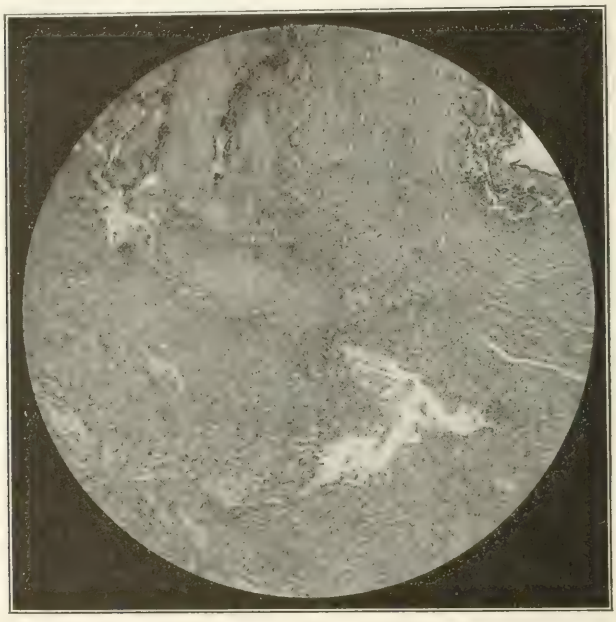

FIG. 13, x 60.—Tubal canal pushed to one side from the proximal pole of the ovum, breaking its way through the muscular layers of the tubal wall.

There is a moderate round cell infiltration of the subserous stratum, which in some areas shows marked edema. In no place does the peritoneum surrounding the sac show any decidua-like cells, or any epithelial invaginations. This stratum is amply provided with a conspicuous network of blood-vessels. The muscular coat presents a distinct division in two strata, the circular and the longitudinal in the sections outside the poles of the ovum, and to some extent at the poles of the ovum; but at the points where the pressure has been considerable, the entire wall is thinned out so as to leave in some spots no traces of muscular layers.

Decidua.—The most important modifications in the gestation sac are noted in the mucosa; I will give particular details of all, tracing them from the sections of the tube outside the proximal pole of the ovum to the sections outside the distal pole.

The proximal side of the tube outside the inner pole of the ovum, probably representing the interstitial part of the tube, shows, as can be seen in Fig. 14 the most remarkable changes of evolution of the mucosa to decidua.

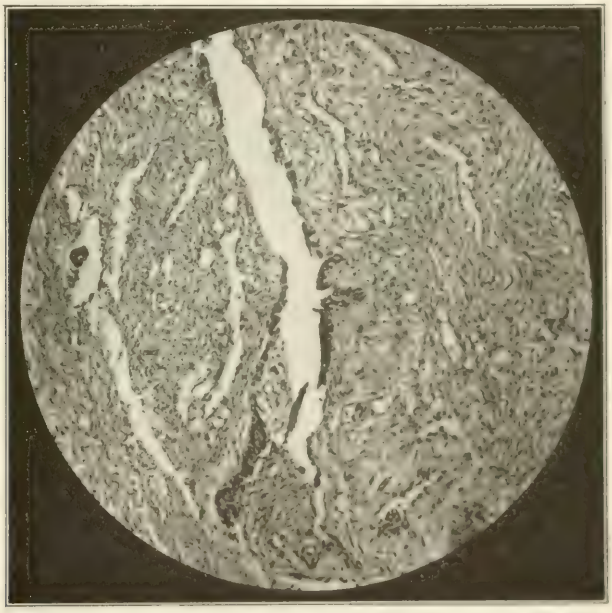

FIG. 14, x 200.—Changes leading to decidual formation in the mucosa outside the proximal pole of the ovum. Flattening and partial disappearance of epithelium. Appearance of decidual cells in the stroma. Great abundance of blood vessels.

The epithelium is partly desquamated; where present, instead of being decidedly columnar, is beginning to assume a flattened shape. In the submucosa, few large round cells with clear protoplasm and nuclei are distinctly noticeable. It is of particular interest to note in the same sections, the glandular like invaginations of the epithelium, and the active proliferation of the endothelium of some blood sinuses in the submucosa stroma.

The next stage of evolution into decidua is well marked as we go further with the sections toward the ovum, and is distinctly seen in

Figs. 15 and 16, one from a drawing, the other from a microphotograph.

The lumen of the tube irregularly shaped and furnished on one side with a few short thick folds, is lined on this side with epithelium, which has assumed a flattened form and is partly desquamating. On the opposite side the mucosa is definitely transformed into a well-developed decidual layer.

The epithelium has entirely disappeared from the surface. The tissue representing the submucous stroma, increases in thickness,

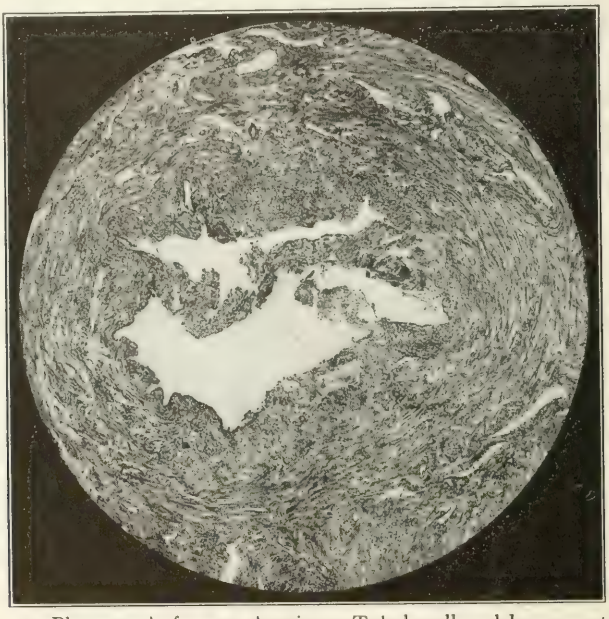

FIG. 15.—Photograph from a drawing. Tubal wall and lumen outside the inner pole of the ovum. The mucosa shows a distinct arrangement in columns of decidua in one side of the lumen. Large spaces at the bases of the columns. In the other side of the lumen the epithelium still present, is entirely flattened.

becomes uneven and bulging from the surface, arranges itself in columns, more or less regular at their edge, presenting at their base large spaces. We have in other words, all the appearances of a well-formed decidua.

The superficial layer, in which some of the columns show beginning degenerative changes, where it is better preserved reveals a marked proliferative activity and the presence of specific decidual cells. These, show almost always a more or less regular round or oval shape, clear protoplasm, and one or two, lightly staining nuclei.

They are diffusely scattered in the stroma, and more noticeable and conspicuous at the base of the columns. The stroma has a very loose appearance, and the cells are widely separated, never as close one to the other as in the uterus.

The spaces of the deep layer are evidently the effect of the fusion of the rising columns at their tops, leaving the bases far apart to constitute artificial cavities.

Going along to the pole of the ovum on the proximal side we find as in Fig. 13 that this occupies a space outside the circular muscular

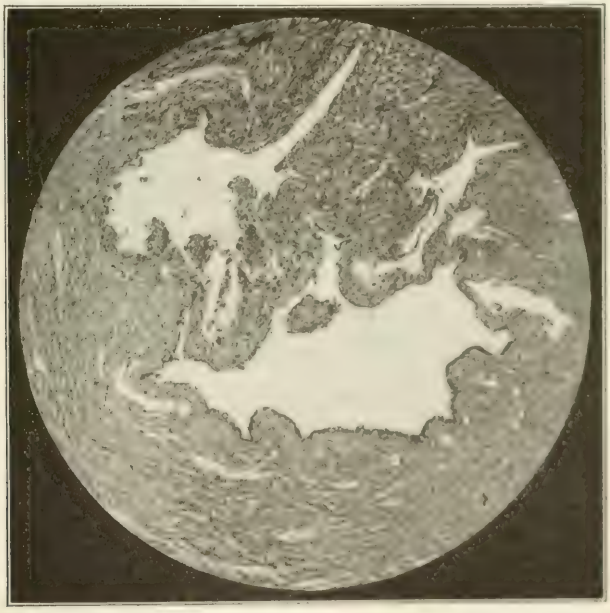

FIG. 16, x 60.—Same arrangement, but more distinct than in the preceding illustration.

layer. At this extreme limit of the implantation cavity, the ovum has not extended through the lumen, which is pushed to one side.

Surrounded by a distinct layer of decidua with the usual columnar disposition and very active, we find in the center organized fibrin and masses and strands of plasmoditrophoblast.

In the lumen the epithelium has completely disappeared (Figs. 17 and 18). Columns of decidua rise almost all around the cavity. The most superficial layer shows distinct coagulation necrosis. The marked invasion of plasmoditrophoblast strands sufficiently explains

the degenerative changes. These strands appear as hollow coverings of villi, infiltrate the spaces at the base of the columns, destroying the primitive connections between compact and spongy layers, and inducing degenerative changes in the superficial structures.

The histological study of the tube outside the external pole does not offer such evident changes as the proximal one. We notice first an hypertrophy of the entire wall mainly in the external layers, longitudinal muscular and connective.

FIG. 17, x 60.—Necrotic changes in the superficial columns of the decidua induced by the action of the tremendous infiltration of plasmodial strands.

The tubal lumen at this point has an irregular star shape in the distal sections, and is provided with from seven to nine short thick simple folds; but going further with the sections toward the ovum, the shape of the canal becomes circular, and the folds gradually disappear, leaving only one at the extreme limit of the pole of the ovum. This fold excessively projecting from the surface dips its stroma more extensively than the surrounding ones.

The epithelium covering the folds and the rest of the lumen is not decidedly columnar; it is partly desquamating, partly proliferating, but can be traced up to the pole of the ovum. The changes in the

stroma, specially that part corresponding to the folds are of particular interest as is seen in Figs. 19 and 20. An abundance of round cells is distinctly noticeable. Some of these round cells are deeply stained, some show a clear protoplasm, and light staining nuclei. Identical but isolated cells intermingled with epithelial cells can be traced to the tip of the folds. The size of the clear cells varies extensively. All of them in fact should be considered as most probable stages of decidual cells.

In fact I will say, that if distinct decidual changes are not evident outside of the ovum in the distal part of the tube, as in the proximal,

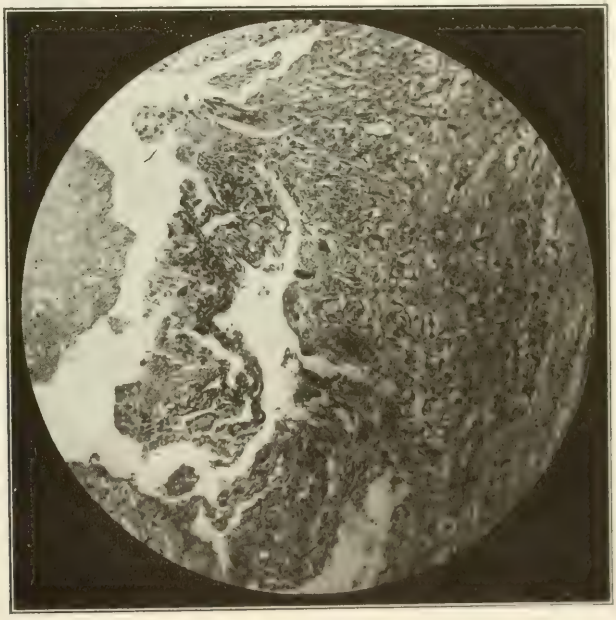

FIG. 18, x 140.—Well-preserved decidua. Plasmodial strands upon its surface.

a clear attempt at preliminary modifications toward that form is certainly noticeable.

The actual pole of the ovum, however, in the distal end evidently shows changes as marked as in the proximal pole. The lumen of the tube, as appears from Fig. 21, is occupied by a structure which has all the characteristics of a well preserved decidua in its periphery probably representing nothing else but the rising of the only fold left and previously described, magnified by the incident decidual transformation at the site of nidation of the ovum.

As we proceed with the sections, the lumen of the tube and the circular muscular layer disappear to give place entirely to the distal pole of the ovum, implanted upon columnar projections of the tubal wall at the section corresponding to the mesosalpinx.

The details offered by the sections at this point are undoubtedly suggestive of being at the site of nidation of the ovum; and notwithstanding the partial alterations induced by the blood extravasation, the topography and relations of fetal and maternal tissue in an early

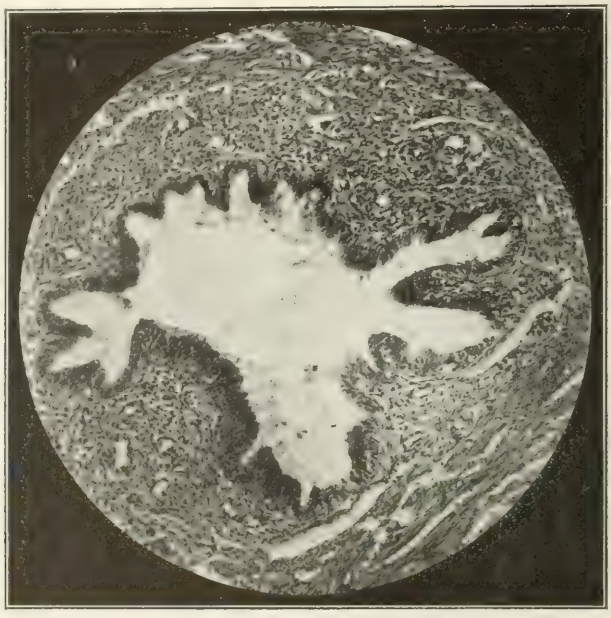

FIG. 19, x 200.—Slight changes in the mucosa of the tube outside of the distal pole of the ovum. Increase of the round cells of the stroma. Partial desquamation of epithelium.

tubal pregnancy, I believe could hardly be found better maintained than in the present specimen.

A simple glance at the entire section as reproduced in Fig. 22, shows a marked distinction in the layers of maternal tissue, which come in contact with the fetal.

What should be considered as the serotina from the evident most intimate relations with the greatest bulk of fetal structures, is represented on the inferior segment of the tubal wall by tissue arranged in short, broad columns, and more or less projecting from

their bases. They are four or five in number in the different sections (Figs. 23, 24, 25 and 26).

From the extreme limit of this projecting columnar arrangement, as is mostly evident on the left side of the section and is better evidenced in the next illustration (Figs. 27 and 28), a fold arises as a capsularis or reflexa to surround the ovum, leaving a distinct space between itself and the inner aspect of the tubal wall upon which the decidua vera lies. On the right side, the excessive pressure from blood extravasation has destroyed the insertion of the capsularis,

FIG. 20, x 400.—Fold of the tubal mucosa outside the distal pole of the ovum, showing epithelial changes. In the stroma abundance of round cells, slightly staining, probable forerunners of decidual cells.

and the end only is scarcely noticeable, adherent to the vera on the tubal wall.

(A) *Serotina and its Relations.*—It is easily divided in two distinct sections, the superficial compact, and a deeper spongy layer, at the base of the columns. The distinction is not to be compared with the one in the uterus. This appearance is only given at this point by the presence of large sinuses in the deeper layer. No gland-like structures are present and there is no epithelium on the surface of the columns. A thin lamella of fibrin, darkly stained with eosin

covers the columns, and the reflexa and vera. This Nitabush layer has to be differentiated from the larger diffuse areas of necrotic decidua, irregularly scattered in the serotina, but specially following the line of invasion of plasmodial masses, although the etiology is probably the same.

As constituents of the serotina we have the characteristic large round or oval, clearly staining uni- or polinucleated cells irregularly scattered throughout the tissue. They are perfectly distinct from the intercellular substance, which is rather homogeneous, faintly

FIG. 21, x 75.—Decidual formation at the pole of the ovum; necrotic in the center, shows in the periphery distinct decidual characters. It is connected by bands to the tubal lumen, which is still lined with epithelium.

fibrillar, but has a lacunar appearance, in which lacunæ the specific cells are situated.

Marked round cell infiltration of the stroma. Marked enlargement of blood-vessels; slight proliferation of the endothelium.

The plasmodium is closely connected with the surface, in some parts it traverses in masses the entire thickness of the columns, and occasionally diffuses into the intercellular substance.

Entire tracts of the surface are covered by the plasmodium, and are intimately connected with the same (Fig. 29).

In Fig. 30, is clearly illustrated the process of invasion of the decidua and blood-vessels by masses of trophoblast. Where the decidua is in direct contact with the traversing plasmodium, it shows marked degenerative changes. At the base of the columns there is an apparent breaking through a sinus by the plasmodium, with consecutive direct contact with the contained blood.

In the details of the same illustration (Fig. 31) it is perfectly possible to follow the process of vacuolization in the plasmodial masses,

FIG. 22, x 14.—Entire section of the tube at the distal pole of the ovum. *Note.*—At the base of decidua, apparently the serotina, surges to surround the ovum as capsularis from the left edge of this fold. On the right side of the illustration such distinction is impossible on account of the blood and fibrin.

consecutive formation of a spongy network, in which meshes the blood rushes from the broken up capillaries of the decidua. It is of great interest to note that at this stage of development the relation of maternal tissue as serotina and formed villi is very occasional and not intimate, and the trophic changes are entirely brought on by the plasmodium, which by invasion of the decidua and blood-vessels brings the blood in the intervillous spaces.

(B) *Capsularis or Reflexa.* This, as seen from Figs. 27 and 28, represents an incomplete attempt at encapsulation of the ovum. In this it succeeds to a certain extent, and were it not for the changes due to the increase in the intracapsular pressure by the growth of

the ovum, and more specially those due to the impending abortion, the attempt would probably appear more perfect.

In fact, in the immediate successive sections, where the pressure has tremendously increased, with no space left in the lumen of the tube, and the blastocyst present with the accompanying fetal structure, it is on the point of being expelled near the break in the tubal wall, all evidence of differentiation of decidua reflexa and vera is obliterated. Aside from this, even where such effort at formation of reflexa is most evident on one side, on the opposite side, however,

FIG. 23, x 100.—Decidual columns of the supposed serotina. Plasmodium covers the surface of the columns and breaks its way through them. It is only occasionally, as in this instance, that villi are close to the surface of the decidua, through buds of plasmodial proliferation.

it is not so distinct, and the reflexa is pushed against the vera by the large blood clot.

As to structure, it is identical with the superficial layer of the serotina. It is provided with less number of decidual cells, and abounds in intercellular tissue with little vascularity. This, and the pressure from the inside structures, sufficiently explain the considerable degenerative changes. The plasmodium is intimately adherent to the inner surface.

The manner in which the fold springs from the serotina, the space between the outer aspect of the fold, and the inner aspect of the remaining tubal wall modified into vera, and their identical structural characters are rather suggestive of this, as being a capsularis or reflexa.

(C) *Vera.*—The vera slightly uneven and various in thickness rests on the tubal muscular layer with no evidence of epithelium on the surface. Here and there a few plasmodial masses can be seen

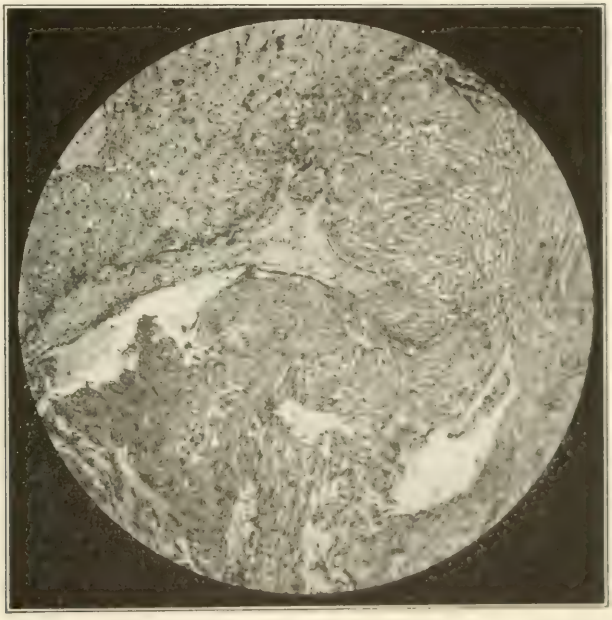

FIG. 24, x 130.—High magnification of the serotina. The constituents are more clearly illustrated than in the preceding Fig. 23.

with a slight tendency at invasion and no evidence of breaking up of the decidua or vessels.

Cells and intercellular substance do not show marked degeneration. There is moderate round cell infiltration.

The most striking feature is the abundance of capillaries and sinuses at the junction of this decidual layer and the subjacent tissue. In some of these vessels, there is a proliferation of endothelium, and no invasion of fetal elements.

Site of Rupture.—Judging from the situation of the supposed serotina on the section of the tube corresponding to the mesosalpinx, the rupture has occurred on the opposite side. What macroscopically

appears as a cribiform structure, microscopically is represented by an active invasion of the tubal wall by villi. The position of the wall of the tube at the site of rupture is thinned out and reduced to a connective lamella traversed by masses of fibrin, in which are entangled villi, some preserved, some degenerated. The edge of each of these small openings is constituted only by the peritoneoconnectival layer on the inner aspect of which are still present in spots groups of decidual cells in a thin layer of evidently necrotic decidua.

FIG. 25, x 100.—Extreme limit of the serotina on the left. Marked distinction of a superficial or compact layer, and a deeper or spongy one. Large sinuses are responsible for this last appearance.

CONSIDERATIONS.

Having presented a description of the case, studied from both the clinical and anatomical side as completely as possible, I desire to conclude with a few considerations, to explain some of the reported data.

The age of the ovum is a problem of great importance in this case. In spite of all the clinical knowledge acquired on the human subject, and through continuous and careful researches in comparative embryology, we possess no secure criteria to estimate the exact age

of the different ova. Our inability to give positive opinions on this question is undoubtedly due to the unsettled relation of menstruation, ovulation and fertilization.

The old theory of Pflüger in considering menstruation, as an immediate effect of ovulation, and the method of calculating the beginning of pregnancy from the date of the last menstruation, was somewhat shaken by the clinical studies of Prochownick and de Sinety, and later by those of Leopold and Ravano. The last authors, from a careful clinical and anatomical study of ninety-five ovaries removed

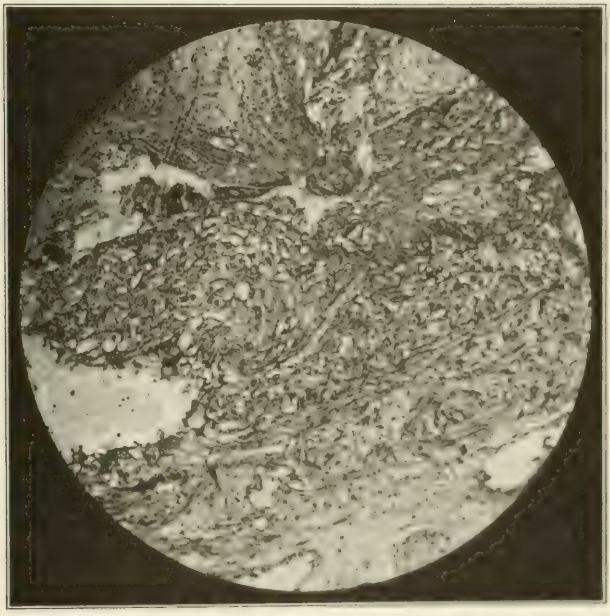

Fig. 26, x 150.—Higher magnification of Fig. 25.

at operations, showing in only fifty-nine (62 per cent.) a synchronism in the two processes, concluded that if in the majority of cases such relation exists, it is, however, often missing, and therefore ovulation and consequently conception may occur at any time.

Even accepting with Teacher and Bryce for the human subject the theory of Heape and Marshall on the œstrum of the lower mammals in interpreting the menstrual cycle, not as far as anatomical changes are concerned, but only as to its bearing on the sexual processes of ovulation and fertilization, we are forced, from the clinical evidences, to recognize the fact that in man the probabilities of conception are

extended with no limitations beyond the boundary of a possible conventional œstrum, corresponding to the postmenstrual period in which the desire is greater.

Teacher and Bryce, fully discussing this side of the question in their "Contribution to the Study of Early Development of Human Ovum," regard coitus as a factor of great importance in determining the age of the ovum when it has occurred only once in the history. Evidently we must accept their contention as the most reliable, but

FIG. 27, x 60.—Reflexa or capsularis, springing as a fold from the extreme left of the serotina. Space between capsularis and vera; this last appears various in thickness on the inner aspect of the remaining constituents of the tubal wall.

even this factor is subject to certain limitations and consequently of relative bearing.

In the first place it is easy to understand the difficulty in securing reliable data on the history of coitus, and how, only through fortuitous circumstances, as in the case of Eternod and the one of Teacher and Bryce, it was possible to do so.

In the second place, we must consider the fact that even in such cases the data may be faulty, if we only think of the possible variations in the travelling of spermatozoon and ovum, of their meeting place, and capacity of living before they meet. In the present state

of knowledge we are not certain of the time the spermatozoon may remain alive in the genital tract and capable of fertilizing before the occurrance of conception. The allowance of twenty-four hours, according to Teacher and Bryce, for fertilization after menstruation, when this last coincides with the discharge of the ovum, is only an assumption, and as such of only relative importance in deciding the age of the ovum.

In view of the uncertainty and consequent insufficiency of the discussed data to establish the correct age of pregnancy, we should

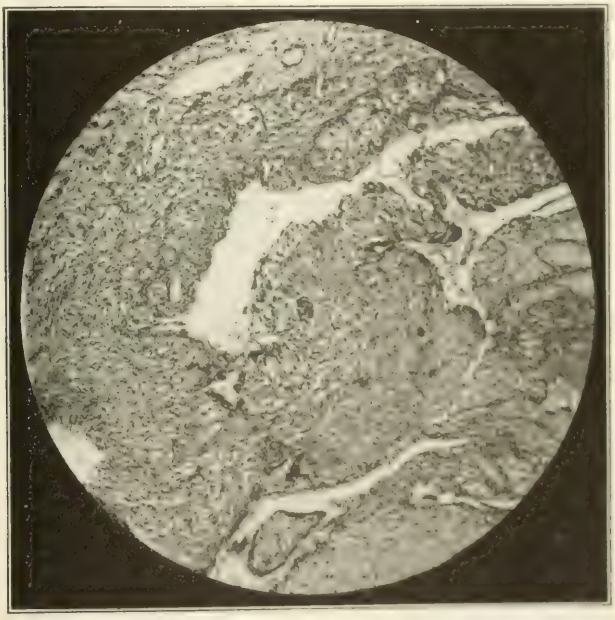

FIG. 28, x 100.—The differentiation of Fig. 27 is shown more distinctly.

rely on the anatomical evidences to secure the best available information. But in spite of the ample knowledge acquired through the direct study of various young human ova, since Peters opened the series of such investigations, we may say that at present the details connected with the first stage of development are still incomplete. Considerable light has been lately brought on the subject by the extensive and comparative study of Teacher and Bryce.

Undoubtedly they have thrown much light on the subject, added new important features and strengthened the chain of anatomical evidence of early human development. They have given very

valuable information on the imbedding of the ovum, and what is of much greater interest, they have made clear the way in which the primary relations are established between ovum and maternal tissue, through the trophospongia, which is similar to that described by Hubrecht for the tarsius. This analogy had been, however, anticipated by Webster in his work on human placentation. They have, besides, endeavored to fix with a most elaborate comparative study, the correct age of the early ova described. On the basis of their findings the ovum of Peters, estimated always to be a few days old, was then considered as thirteen and a half or fourteen and a half days.

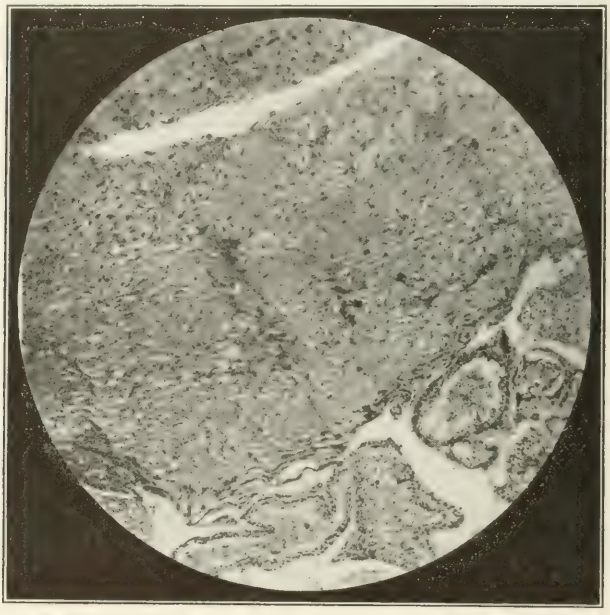

FIG. 29, x 60.—Close relations of plasmodium and decidua.

Strong points of differentiation, according to Teacher and Bryce, are furnished apart from the embryo, which may be absent, as in some of the specimens studied, by variation in the features of the primitive trophoblastic formations by the state of the blastocyst and characters of the mesoblast.

In comparing the three supposed youngest ova, the one of Peters, the one of Leopold and their own on the basis of their findings (undifferentiated condition of the chorion of the blastocyst, absence of a cellular layer in the trophoblastic process, absence of arrangement

of the thin mesoblast in a denser layer around the chorionic wall, and absence of identations of the wall), they tabulate them as follows: Teacher and Bryce, the youngest, Leopold next, and Peters the oldest. In fact, the last is characterized by a more distinct state of differentiation in the two varieties of cells, either in the blastocyst or in the trophoblastic buds, by some outgrowth of mesoblast in the trophoblastic depressions, and a more definite intermingling of fetal elements and decidua.

But due consideration must be given to the variations of development or to some pathologic conditions of the ova studied. This

FIG. 30, x 100.—Plasmodial masses and cytotrophoblast breaking through decidua and sinuses.

fact, well appreciated by Teacher and Bryce, was long anticipated in the statement of Webster in his study on human placentation, that early ova are subject to considerable variations in regard to the rate of growth, shape, appearance and rapidity of development of villi. Therefore, we must conclude that, until many careful observations of early normal specimens have fixed the anatomical data relative to the different stages of evolution, we shall not have a positive rule by which to estimate the exact age of the ovum. In an early tubal ovum, as the one described, the above mentioned diffi-

culties are accentuated by the fact that the tube is an improper ground for the development of the ovum. Disturbances in the normal evolution and pathological conditions are rather liable to occur, thus altering the normal features. For instance, absence of the embryo in the tube should be considered as a frequent occurrence, so that this very same absence in my case, instead of being due to the failure to secure serial sections, was probably due to trophic disturbances. This assumption is borne out by the relative disproportion between the blastocystic formation and evolution of villi.

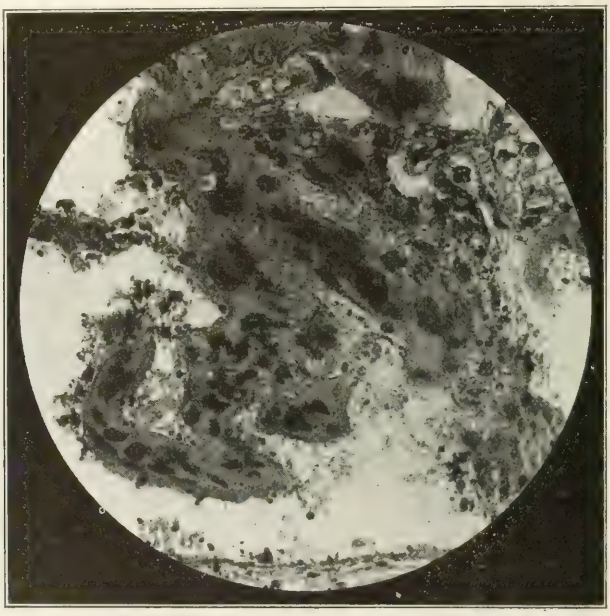

FIG. 31, x 475.—Plasmodial masses on the surface of the decidua vacuoles in which rushes the extravasated blood.

It is a well-known fact, on the other hand, that normal development of villi is possible in the absence of the embryo, and consequently with interruption in the growth of the blastocyst.

In view of all these evidences the only reliable factor for the determination of the age of this ovum is practically the date of the last menstruation. In this regard, Dr. J. Whitridge Williams, to whom I submitted microscopical slides of the specimen writes: "There is no doubt that you had to deal with a very early tubal pregnancy, and that the structure in the center of the specimen represents the blastocyst. Further than this I should not be willing

to say on account of the absence of the embryonic area. I think, however, in view of the history of the case, that you are safe in saying that the pregnancy is not older than three weeks."

The history further allowing at least a week after the last period, supports this contention, more than all the recorded anatomical features, by the relation of fetal and maternal structures. These in fact consist only of lightly intermingling plasmodial masses and decidual columns; at no point does any villus break into the decidual columns.

The size and broken connections of the ovum and implantation site are responsible for the loss of all evidences of the manner of imbedding, and the site of imbedding can only be approximately estimated.

On the contrary, we have enough definite features regarding the formation of the decidua to convince us that there is no reason to believe that this process is any different in the tube from that in the uterus. Undoubtedly we have to allow for some variation on account of the anatomical peculiarities of each of the two mucous membranes.

Absence of glands in the tube could easily explain the absence of a definite spongy layer; but even in this respect, as we have seen, Nature attempts almost an identical process although infrequently, for the inclusion at intervals in the proliferating stroma evolving to decidua of cavities lined by epithelium, representing the fundus of the tubal folds.

Prevalence of the intercellular fibers in the stroma of the tubal mucosa accounts for the sparsely grouped cellular appearance of the tubal decidua, varying from the uterine, in which the cells are closely packed.

After a careful analysis of the reported cases, I feel safe in stating that none of them show such remarkable arrangement of decidua extending continuously from one pole to the other, only thinned out in the equatorial zone of the ovum at the points of greatest pressure.

The details as above described with the illustrations as given, should be sufficient to dispense with further remark. But the importance of the question, and the doubt cast upon it by many observers, as Couvelaire, Bandler, are sufficient to justify any explanation of the data found.

The most typical changes, leading to the production of decidua are clearly shown in the sections of the tube outside of the pole of the ovum.

Disappearance of the epithelium, increase in number and modifica

tion of the round cells of the stroma, gradually developing into specific decidual elements, typical arrangement in columns or round elevations of the modified stroma, abundant network of blood-vessels and sinuses, all these represent an acutal evolution of the tubal mucosa to a decidua vera.

When we consider that these features are supplemented by a definite attempt to differentiate the decidua into serotina, reflexa and vera, as described and illustrated at certain points, we must conclude that the process of decidual formation is identical, with slight variations, in the uterus and in the tube.

It is logical to assume that the receptivity for imbedding the ovum in tube and uterus is identical. That is, the response of the tissues to the same stimulus must be teleologically consonant. In other words, if the decidua plays an important rôle in the early process of pregnancy, there is no doubt that a similar formation is essential wherever there is a possibility for pregnancy.

This mode of interpreting the formation of decidua as a necessary effect of pregnancy is evidently at variance with the theory of Webster, which attributes to the uterine mucosa in man the normal faculty of forming decidua, and only as a result of faulty development it is occasioned to the tube and a cause of ectopic. But this specificity accorded to the Müllerian tissue, and its interpretation as the etiologic factor of ectopic have been discredited by the undisputed findings of ovarian pregnancy, and decidual formations in other organs (appendix, peritoneum) during pregnancy. On the other hand, we are forced to discard on the basis of the advocated theory, the belief that the decidua menstrualis is a possible monthly preparation for the reception of a fecundated ovum. Great support to this contention is found in the fact that anatomical differences exist between the process of decidua menstrualis and the earliest decidua of pregnancy. One is attended by partial disintegration and consecutive regeneration, the other, as far as we know, from its inception, is entirely a formative process. Even recognizing with Adler and Hitschmann that during the congested premenstrual swelling, endometrium closely approaches that of an early decidua, the well-known fact that the greatest number of pregnancies are estimated to occur after the last period, makes the two processes entirely distinct.

Taking now into consideration the formation of the reflexa, the tube, as Webster has pointed out is not as apt as the uterus to cause its development.

According to the latest accepted idea on the imbedding of the ovum in the uterus, suggested by the important investigations of Hubrecht

on the hedgehog, Graf. v. Spee on the guinea-pig, Peters first and Teacher and Bryce most recently on the human subject, the reflexa or capsularis of His is constituted by the blending of the lips of the growing decidua at the extremities of the depression in the stroma, formed by the ovum eating its way through the modified mucosa deprived of epithelium (serotina). Given the size of the uterine cavity plenty of space is left between this decidua arising and surrounding the ovum, and the modified mucous membrane lining the rest of the cavity (decidua vera).

The fusion of the capsularis with the vera, and consequent disappearance of the intermediate space, is only a later occurrence. In the tube, on account of the small size of the canal, immediately after the implantation, or shortly after, the ovum occupies the entire cavity, offering little or no chances at differentiation; and the reflexa is rapidly crowded against the vera, becoming fused with it or necrotic. Notwithstanding this unfavorable condition for its development found in the tube, the reflexa has been reported to be apparently well formed as in the specimen mentioned by Webster.

In my specimen many sections on the outside pole of the ovum suggest an attempt at formation of the reflexa. Absence of epithelium on the outside aspect of this structure would be a reasonable objection against considering it as such; but in this specimen, even on the section outside the ovum wherever there is considerable evolution of the mucosa to decidua, the epithelium has disappeared.

I deem it necessary to emphasize the fact, that at this rather early stage the relation of fetal and maternal elements is almost exclusively and sufficiently established by an intermingling of the plasmodial elements within the decidual columns. The Langhans cells are comparatively few and rather grouped with the plasmodium which seems to play mainly the rôle of breaking through the decidua and decidual sinuses to secure the trophic changes for the ovum.

No villi are deeply situated, but only seem to be close to the tips of the decidual columns, or attached to them by bands of proliferating cytotrophoblastic elements.

In a more advanced stage of tubal pregnancy, at the fifth week, the relations are almost the opposite. Extensive proliferations of the cellular buds are extending through the decidual columns, paving the way to the villi, breaking through the decidua and the sinuses.

Even where the Langhans cells are most diffused, their smaller size and better affinity for the stain are sufficient to differentiate them from the characteristic decidual cells.

I wish to extend now my sincerest appreciation for the suggestions

and encouragement afforded me by Dr. Ewing, Director of the Pathological Department of Cornell University, to Dr. Stockard, Professor of Anatomy at Cornell University, to Dr. J. Whitridge Williams, of Johns Hopkins University, for the interpretation of some of the sections, and to Mr. Dunn, of the Loomis Laboratory, for his microphotographic work.

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OVARIAN PREGNANCY, WITH REPORT OF A CASE.*

BY

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(With thirteen illustrations.)

Up to a few years ago the best authorities on ectopic pregnancy refused to recognize the possibility of ovarian gestation, and the cases reported as such were not sufficient to really warrant their acceptance. Tait, Sutton, and Webster emphatically denied the fertilization and implantation of the ovum in the ovary. The last author based his opinion on two points.

First, the insufficient evidence in the cases described.

Second, the assumption founded on his theory of the decidual reaction that the implantation and development of the human ovum is possible only in tissue derived from the Müllerian tract, and consequent inability of the ovary to develop such specific reaction. (Webster, *Ectopic Pregnancy*, Edinburgh and London, 1895.) Kower (1893)[1] presented before a gynecological society a specimen of ovarian pregnancy, a summarized pathological report of which was later published by Dr. Catherine Van Tussenbrock (*Centralblatt für Gyn.*, 1897). This in fact is the first positive case on record, and was later (1899) thoroughly described and carefully studied anatomically by Dr. Van Tussenbrock. The complete description in detail of the Van Tussenbrock specimen, besides proving beyond doubt the real condition, and disproving the statement of Webster, served as a guide for further observations. For a long time convincing data had been demanded as evidence of primary ovarian gestation, but the opinions of observers differed as to the necessary requirements.

Spiegelberg as far back as 1878 was the first to suggest certain factors of identification, viz.:

(*a*) Integrity of the tube on the affected side.

(*b*) Fetal sac in the position of the ovary.

(*c*) Connection of the sac with the uterus by the ovarian ligament.

(*d*) Ovarian tissue distinctly demonstrated in the sac wall.

*Read before the Gynecological Section, New York Academy of Medicine, December 23, 1913. Histological Work from the Pathological Department of Cornell University.

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These requirements were later considered insufficient. Heinecken insisted on the presence of the placenta inside the ovarian tissue. Williams added to the demands of Spiegelberg that ovarian tissue should be found in several distant parts of the sac. Werth requires the corresponding tube to be absolutely intact and independent from the gestation sac. Norris making more complete this last requirement, demands that the tube on the affected side should be microscopically negative of evidence of pregnancy. Upon this basis the most complete and careful study of the specimen should decide as to the condition. But if we consider the different opinions as to these criteria, the difficulty in having them all supported by the actual find-

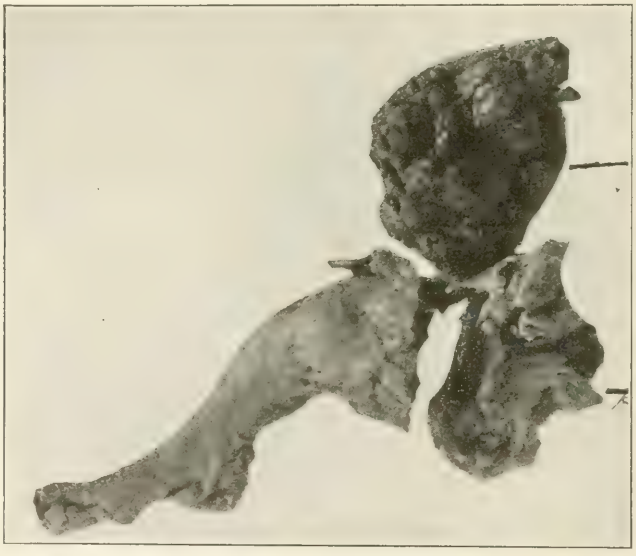

FIG. 1.—*A*. Distal pole of left ovary, site of rupture. *B*, Fimbriated extremity free from adhesions, open.

ings, and the rare presence of all of them in the same case, we will easily understand how seldom the diagnosis can be satisfactorily established. All the requirements, however, seem to be lacking in something. That of Norris, for instance, which stands as the most complete, is still open to certain criticism. Can he doubt the primary development of the gestation in the ovary, because of finding of a decidual reaction in the corresponding tube? If with a tubal pregnancy the opposite tube is apt to react, why cannot we obtain the same reaction in the tube connected with the pregnant ovary? In his case of combined ovarian and uterine pregnancy he found de-

cidual reaction in the proximal third of the tube and quite correctly ascribed it to the actual uterine pregnancy. However, at present the material at hand is so scant as to make premature and hazardous any conclusion. As to anatomical relations of tube and ovary, how can we recognize them, when the gestation sac is too far advanced, has flattened out all the surrounding tissues, and has formed adhesions, reducing to a shapeless mass tubal and ovarian structures? How can we establish anatomical relations when the operation is performed several days after the occurrence of the rupture? Webster has reviewed the many instances in which a mistake is possible, viz.:

FIG. 2.—The inner aspect of the resected ovary from which sections were made.

(*a*) Hematoma ovarii.

(*b*) Pregnancy in an accessory tube in which case the sac becomes closely adherent to the ovary, and the normal tube is found free from the gestation sac.

(*c*) Pregnancy in an accessory fimbriated extremity, in a diverticulum of the tube, or on the ovarian fimbriæ in which the ovary may become such an intimate part of the sac as to be mistaken for the primary seat of implantation of the ovum.

(*d*) Pregnancy in the fimbriated extremity of the tube, the fimbriated extremity being in this case adherent to the sac.

(*e*) Intraligamentous (and to this I would add abdominal pregnancy) secondary to tubal abortion or rupture with consecutive intimate adherence to the ovary, as in a case carefully studied in which Webster could trace the site of the rupture in the greatly distended tube which was adherent to the sac, but apparently independent of it.

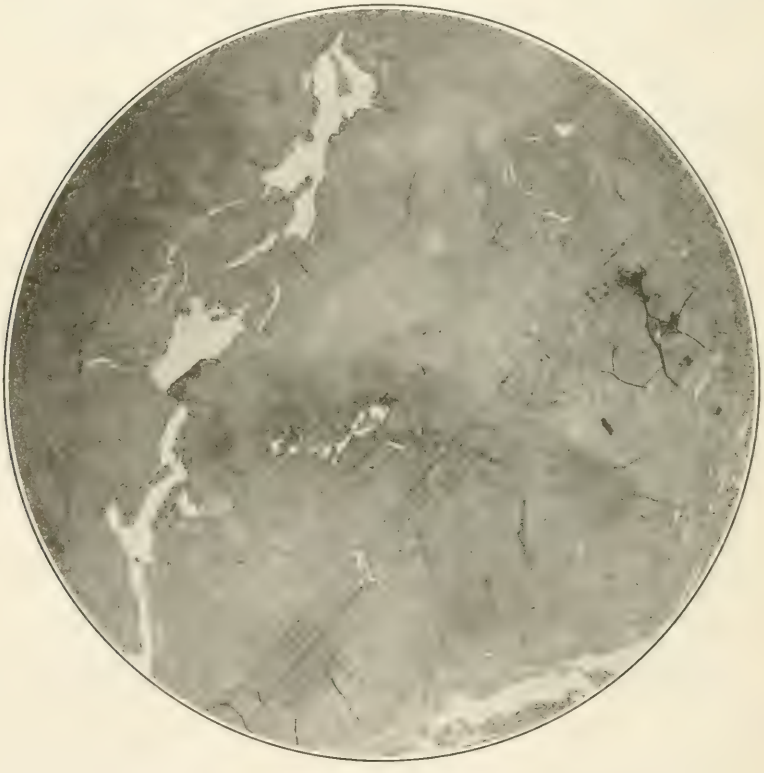

FIG. 3.—Section showing corpus luteum and ovarian stroma.

All these possible causes of error are more apt to occur, the more advanced the pregnancy. No doubt in the earlier cases the task is comparatively easy. The anatomical relations are less altered and the microscopical findings show such intimate connection of fetal and ovarian tissues, that there is hardly any doubt as to the origin of the pregnancy.

One factor of great importance in ascertaining the ovarian origin

is found in the statement of Van Tussenbrock, that ovarian pregnancy means pregnancy in an ovarian follicle. This statement holds good, sustained as it is, by the evidence found in the statistics. Eleven out of nineteen cases reported by Norris showed evidence of corpus luteum in different stages of evolution, connected more or less closely to the gestation sac.

The situation of placental tissue deep down in the follicle and consequently enclosed and surrounded by ovarian tissue, cannot be con-

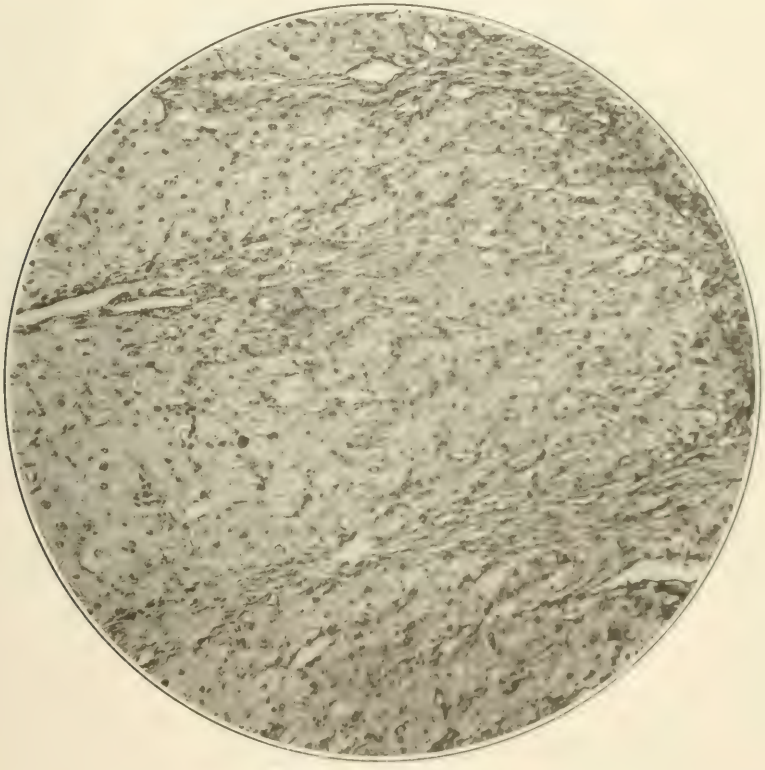

FIG. 4.—Corpus luteum.

sidered as a secondary invasion of the ovary or possibly be mistaken for a simple adhesion of tissue.

The exclusion of any evidence of pregnancy in the corresponding tube (fetal elements) is a strong point in favor of the ovarian origin. Decidual reaction is perfectly possible and could not be considered as strong negative evidence against the diagnosis of ovarian pregnancy.

We must conclude that in early cases, the diagnosis of primary

ovarian pregnancy is relatively easy after a careful combined microscopical study of the specimen.

On account of the great difficulty of determining the real nature of the cases Williams divides them into positive, highly probable, probable and doubtful. Up to 1906 he was able to find only thirteen cases which belonged to the first class, and seventeen to the second.

Norris in 1909 reported nineteen cases. Then follow the reports

FIG. 5.—Relation of fetal tissue with column of corpus luteum.

of Bryce, A. W. W. Lea, Rubin, Graham (1912), and McCann (1913), each having had one case.

Etiology.—The rare occurrence of the condition makes the real causes of ovarian pregnancy rather obscure, more so on account of the unsettled opinion as to the determining causes of ectopic pregnancy in general. But obscure as it is, the etiology is always the most interesting question connected with the study of this subject.

Those ingenious hypotheses which Webster presented as a foun-

dation, in accounting for tubal pregnancies, did not extend to the explanation of those ectopics occurring outside the tube.

He insisted on the decidual reaction as an indispensable factor to the implantation of the ovum, and believed this reaction to be specific to Müllerian tissue only.

In the human female, under normal conditions, only the uterus is able to respond to the genetic influence. The tube occasionally

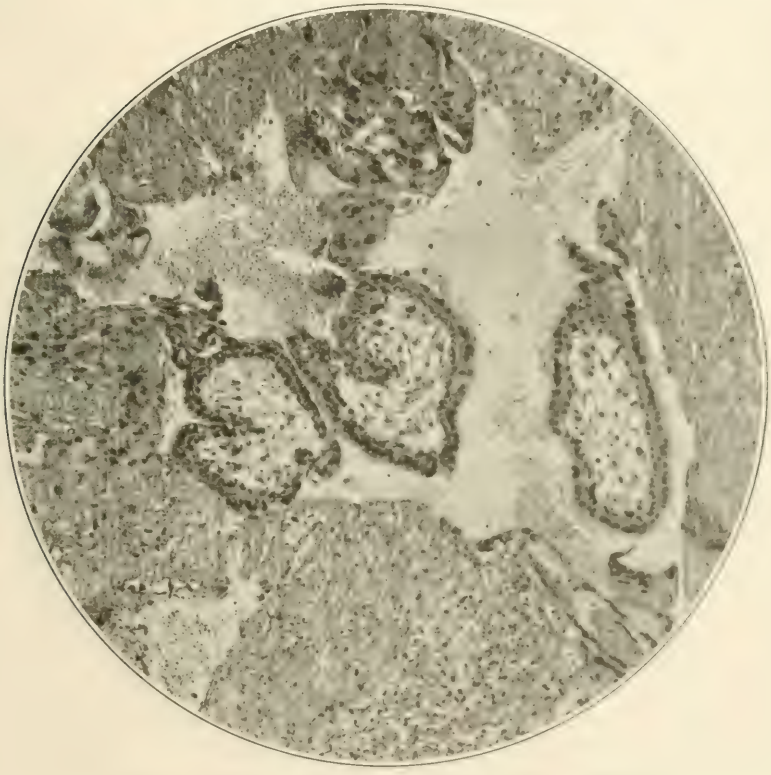

FIG. 6.—Villi showing distinctly both epithelial strata.

reacts as a result of some developmental fault, or as a structural or functional reversion of the tubal mucosa to an earlier type in mammalian evolution.

When these conditions exist, several contributory causes (site of fertilization above or below the point of occurrence of the decidual reaction in the tube, inflammatory processes, distortions, tumors in the tube) will determine whether a fertilized ovum shall develop in the tube or in the uterus.

As a logical consequence of these views, Webster, at the time of the publication of his theory, refused to accept the possibility of ovarian pregnancy with the statement that there can be no pregnancy where no genetic reaction is possible. There is no reason to believe that the Graafian follicle can respond to the genetic influence, and there is no proof that any pregnancy ever started in them.

Now, without going into all the details, and successive studies of

FIG. 7.—Chorionic villi completely surrounded by columns of lutein cells.

the formation of the decidua in the uterus and tubes, I will confine myself to the statement that it is the general consensus of opinion that decidual reaction in the human female is only specific to the uterus in consequence of the functional requirement and occasionally occurs in the tube, but is never as complete as in the uterus. There is no reason to believe that this reaction is a primary cause of ectopic in the tube, but it is more logical to regard it as a result of the specific action of the fertilized ovum in a tissue homologous or phylogenetic-

ally analogous to the submucous layer of the uterus. We should also remember that ectopic pregnancy is a pathological condition, and as such, only pathological factors are worthy of consideration as etiological.

Reversion of function is only a vague theoretical point, when it has no anatomical ground as a foundation. Due importance must be given as efficient causes to all the conditions considered by Webster

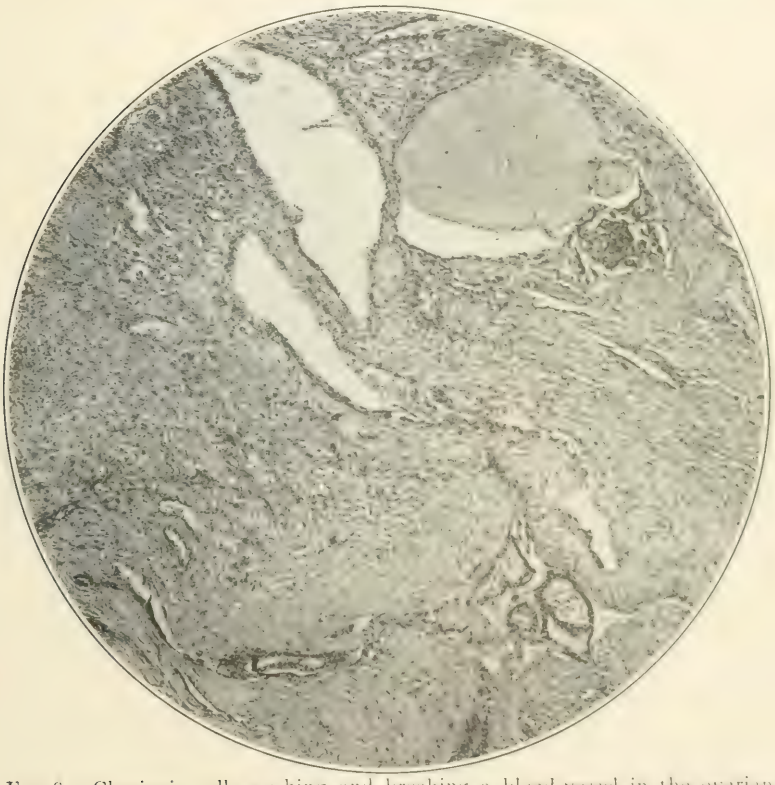

FIG. 8.—Chorionic cells pushing and breaking a blood-vessel in the ovarian stroma.

as only contributory. In considering now the data acquired through a careful study of the reported cases of ovarian pregnancy, we feel inclined to state that the definite acceptance of them is a strong argument against Webster's theory.

When Webster himself published his first case he was forced to recognize the occurrence of ovarian pregnancy, but to support his theory he ascribed the condition to the inclusion of Mullerian tissue in the ovary.

The researches of Williams and the positive findings of columnar epithelial structures in the ovary could not be considered sufficient ground for this contention. Presence of Müllerian tissue in close relation to the fetal structures should be essential to maintain this claim.

In none of the reported cases of ovarian pregnancy, however, has such occurrence been verified, and in the only instance, recently

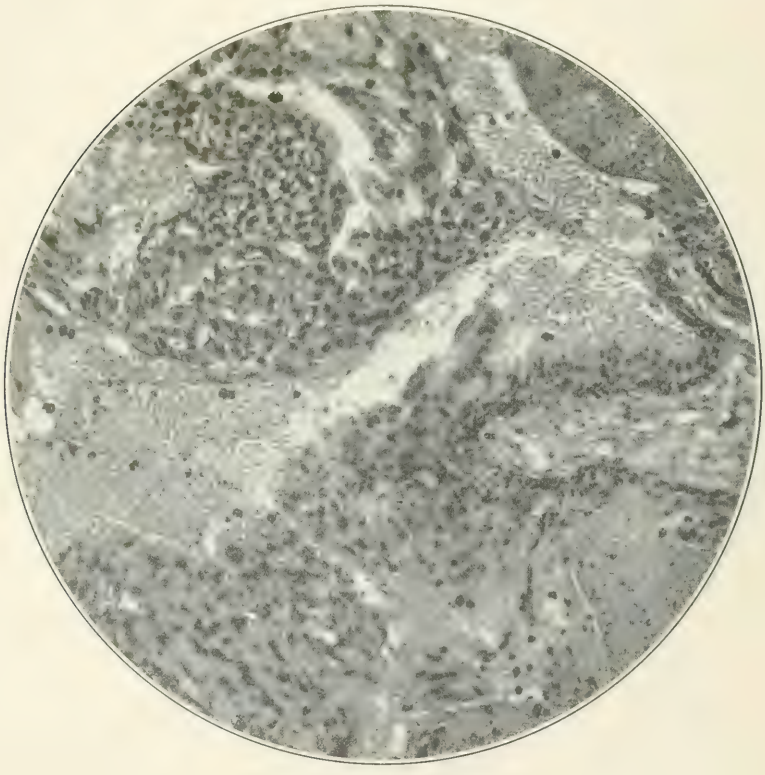

FIG. 9.—Villi and proliferating epithelial layer.

reported by Graham, in which a tubule with epithelium and muscular tissue was present, the structure was quite distant from the site of the ovum, and did not show any changes indicating previous relation with the ovum. This is in opposition to the contention of Graham, that the ovum was primarily implanted in that tubule, and secondarily was attracted toward the corpus luteum.

Besides the presence of decidual changes found by Walker, Dobbert, Schwartz and others in the pouch of Douglas, and at many

other points in the subserous tissue surrounding the uterus, and also in other structures as vagina, parovarian cysts and omentum and appendix (in this last structure quite recently by G. Outerbridge), could not easily be explained with the same inclusion theory. On the other hand no decidual reaction of any considerable extent has been found in the pregnant ovary, except a slight one recorded by Webster in one case and Rubin in another. The second complete report of Van Tussenbrock rejects the acknowledged decidual reac-

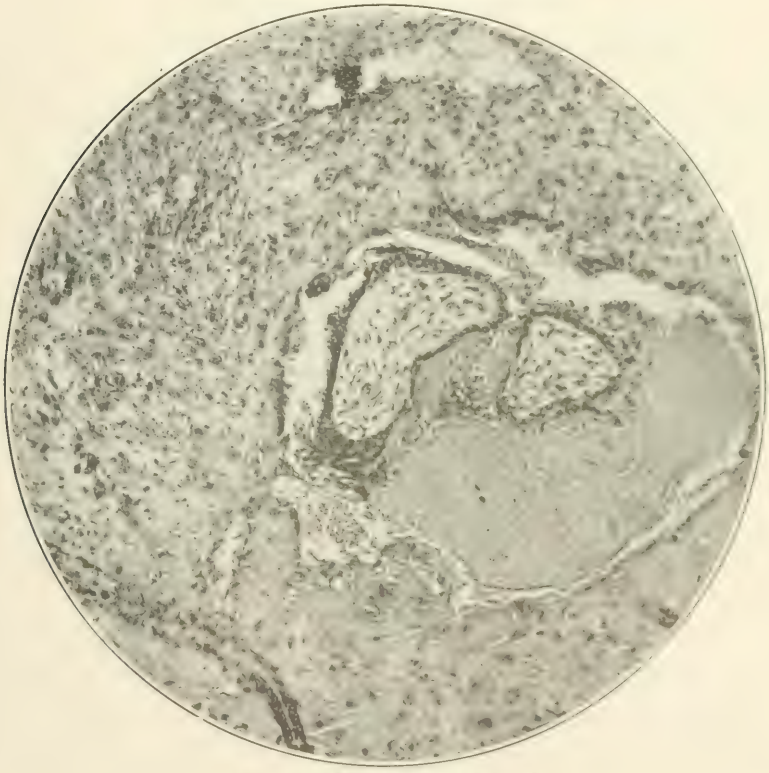

FIG. 10.—Villi in a large sinus.

tion of the first report and after most careful differential analysis she recognized the lutein nature of the cells.

The necrotic changes incident to the tissue surrounding the ovum and the difficulty of differentiating the two varieties of cells was responsible for the confusion.

Bryce and Teacher acknowledge the presence of decidua-like cells in the ovarian stroma in their specimen.

In my first report, which accompanied the presentation of my specimen to the Pathological Society, I spoke of a slight decidual reaction in my specimen.

After the most careful study of various sections with the assistance of Dr. Ewing, I found what I would call an attempt of decidual formation. Here and there, scattered groups of cells of decidua-like nature were found, especially around blood-vessels. In no part of the fibrillar connective tissue covering the columns of lutein cells

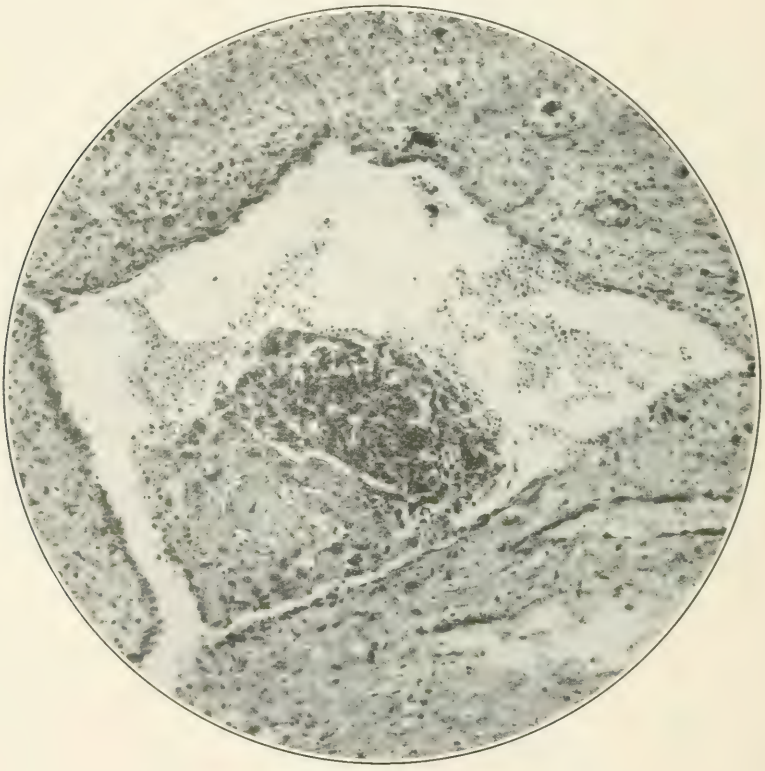

FIG. 11.—Chorionic cells free in a large sinus.

or the fibrous septa of the same columns, considered by Tussenbrock as the tissue only possible to react, was there any evidence of decidual cells.

Tussenbrock claims that the absence of decidual reaction in the ovary would easily explain, from the lack of the necessary trophic properties of the decidua, the hemorrhages between ovular sac and material tissue. If we consider the decidua as a barrier to the inva-

sion of the fetal tissue, we can easily explain from its insufficient rmation the considerable invasion of villi and chorionic cells in the ovarian stroma and in parts quite distant from the fetal tissue.

These pathological facts stand against the statement that many cases considered as ovarian went to term, probably on account of the greater accommodation of the ovarian tissue.

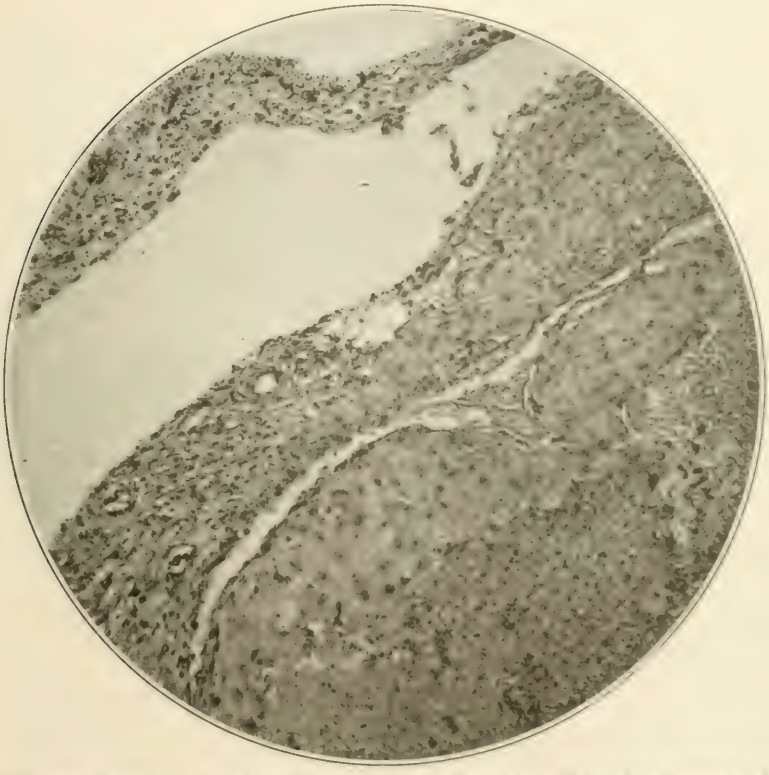

FIG. 12.—Layer of decidua-like cells in the boundary of ovarian stroma and fibrin enclosing fetal structures.

Ovarian pregnancy, until it was clearly demonstrated, was considered impossible, as the ovary could hardly be thought of as a possible meeting place for the spermatozoon and ovum. Stratz observed in the Sorex the penetration of the spermatozoon in the follicle between the cells of the granulosa. In the human female this is hardly possible of demonstration. But, if we have to reason by analogy, and on the other hand if we have to accept as primary, the implantation of the ovum in the ovary, we cannot but admit that the

fertilization was also ovarian. Eleven cases out of nineteen reported by Norris showed a close relation of the ovum with a corpus luteum.

Leopold admits that the ovum from a follicle deeply situated passes into a superficial one, in which it becomes fecundated by a spermatozoon which had penetrated there.

This hypothesis has had no actual proof. Clinical data, supported by anatomical findings, and the opinions of the majority of authors

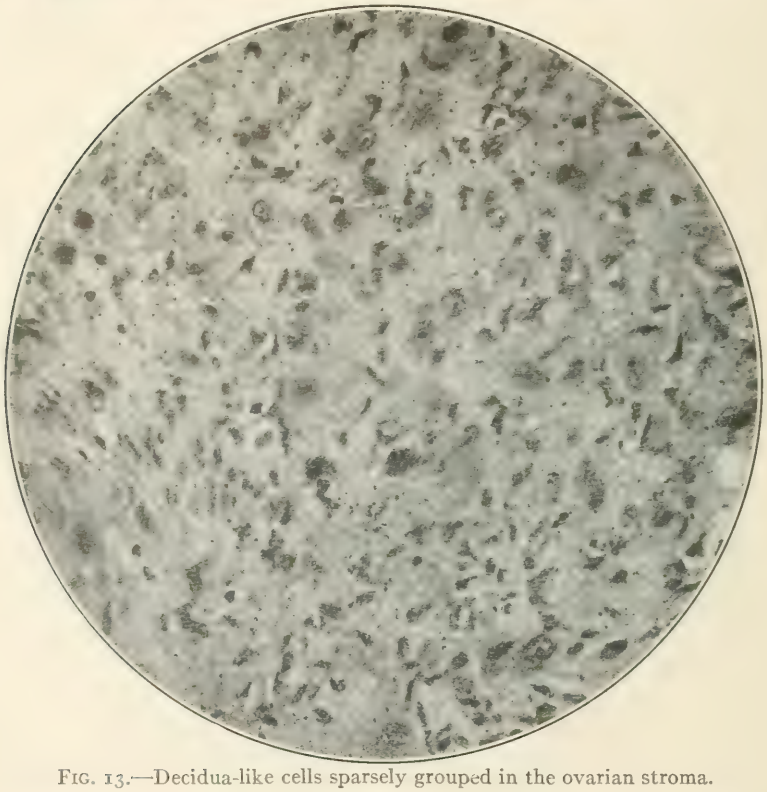

FIG. 13.—Decidua-like cells sparsely grouped in the ovarian stroma.

tend to attribute great importance to inflammatory processes of the tubes as etiological factors of tubal gestation.

Inflammations of the ovary, and especially periovaritis are most probable causes of ovarian pregnancy. The resistance of such ovarian tissue to rupture by the ripening follicles and the subsequent formation of cystic conditions is familiar to us all. It is quite possible that the opening in the mature follicle may be insufficient to allow the exit of the ovum, or incidental disease of the granulosa or of the theca folliculi render impossible the escape of the ovum.

In my case the patient had been infected with gonorrhea. As a result she had a tubal ectopic on the right side two years previous to this ectopic, which occurred in the left. Evidence of inflammatory processes are clearly shown in tube and ovary.

As to symptomatology and clinical diagnosis the condition does not offer any special interest, in no way differing from tubal ectopic. A brief history of the case follows herewith.

M. C., thirty-three years old, Italian. Menstruated at fourteen years, regular. Married twelve years, three children. The last five years ago. Five miscarriages; the last one four years ago. Infected with syphilis and gonorrhea. Acute attack of gonorrhea fifteen months before first ectopic. In June, 1909, I operated on for ectopic in the right tube. Tube and ovary of right side were her removed.

In January, 1912, she missed her period for a few days. On the 10th of January she had a severe attack of pain all over the abdomen accompanied by scanty flow. In the next fifteen days she had two or three more successive attacks with collapse. Was treated at home with ice-bag on the abdomen until the real condition was diagnosed. When I saw her on January 24, I noted abdomen moderately distended and tender. Left side of pelvis tender and rigid; no distinct mass could be detected by abdominal examination. Vaginal examination showed uterus in good position. A distinct mass on the left side. Posterior culdesac tender and little distended. No evident passage of membranes had occurred. Slight dark bloody discharge.

At the operation, fifteen days after initial attack, the abdomen was moderately filled with free and clotted blood. Clots around left adnexa, but no formation of a distinct hematocele. Fimbriated end of tube open. No trace of rupture in the tube. The ovary on the distal pole was apparently damaged and covered by clotted blood. The case was considered at the time as one of tubal abortion. The tube was removed and with it almost a third of left ovary was resected on the distal pole. This resection was necessary on account of the absence of the right ovary, removed at the previous operation. The condition of the ovary was interpreted as one of hematoma from rupture at the site of the follicle. The real nature of the specimen was not found until microscopical examination was made.

Gross Specimen.—It is made up of the left tube and part of the left ovary still attached to the tube by a slight band of tissue. The tube, about 7 cm. long, was cut very close into the uterine horn. No marked enlargement at any point. The ampulla was thickened and hard. No kinks, no traces of rupture.

The fimbriated extremity was free from adhesions, but traces of blood were found adherent to some fimbriæ and external surface of the tube.

A section 7 mm. thick comprising part of the ampulla was cut

out for microscopical research, leaving the fimbriated extremity detached.

The resected ovary was reduced in the specimen to an oval flat section measuring 2 by 3 cm. in diameter and about 5 mm. thick, representing the distal pole of left ovary still connected to the tube by a slight band of tissue. The outside surface apparently rough, shows a central dark area surrounded by a thin layer of grayish tissue. All this surface represents the site of the rupture.

The photograph is not complete on account of the pieces cut out for the microscopical sections.

The contrast between the central and the peripheral area is most marked on the inner surface.

A portion of tissue, 7 mm. thick, was cut from the middle of the specimen of the resected ovary, leaving the inner end which macroscopically shows the structure of a corpus luteum.

From this ovarian section, from the section taken from the tube, and from the corpus luteum many slides were made for microscopical study.

In comparing the gross appearance with the microscopical findings the embedding of the ovum seems to have been deeply situated about 1.5 cm. in the ovarian substance.

Microscopical Findings.—The tissue was fixed in 10 per cent. formalin and embedded in paraffin. The sections were stained with hematoxylin-eosin. One microphotograph (magnified 20) was taken of one entire section, comprising almost all the area of tissue. In this microphotograph we can easily distinguish a peripheral layer of tissue more or less loosely connected with the central area of tissue which is broken up in many points.

In one segment of the peripheral layer, the characteristics of the corpus luteum are clearly apparent from the inner aspect and almost intimately connected with the same is a triangular-shaped section of tissue. This section assumes well the hematoxylin stain and from the details to be later described could possibly be considered the placental implantation.

Going now into the microscopical details, we find the surrounding ovarian stroma reduced to a thin shell, except where the corpus luteum is situated.

In none of the sections could I find any trace of germinal epithelium, no follicles in any stage of evolution, and no corpora albicantia or cysts. The ovarian stroma was apparently normal. No evidence of recent or past inflammatory processes. What is most striking is the abundance of blood-vessels in this cortical layer. Large veins are filled with blood. In the parts in which the corpus luteum is absent the ovarian stroma is exceedingly thin and loose. The tissue seems to be thinned out by the eccentric pressure of the extravasated blood, but due consideration must be given to the active invasion of fetal elements. In these parts of the stroma in fact, and not in any of the parts externally contiguous to the corpus luteum, the invasion of villi and chorionic elements is considerable. Large veins contain villi and chorionic elements free in the lumen,

and in some sections we can easily follow the process of the fetal elements pushing and breaking through the walls of blood-vessels to penetrate them.

A part from this external layer and 2 mm. inside the surface lies a well-formed segment of a corpus luteum. It consists of from four to six festoons in the different sections.

In some of the sections the concave side of the corpus luteum faces toward the central area, is intimately connected with the fetal tissue, and its extremities extend directly into the ovarian stroma.

In one section the corpus luteum formation is traversed by fetal tissue invading ovarian stroma.

In another section probably at the extreme end of the fetal implantation, the corpus luteum forms a ring surrounding a cavity filled with fetal elements.

This structure is well developed. It plainly shows the festooned appearance, due to the distribution of the large epithelioid cells, with granular protoplasm, and small round faintly staining nuclei, traversed by fibrous partitions.

These septa are continuous with the connective tissue forming the external theca, and with the thin fibrous layer covering the inner side of the corpus luteum. Many blood-vessels and lymphatics are present in these fibrous partitions. The lutein cells which are almost all normal, seem in the highest stage of evolution, and only few show degenerative changes.

The central area is constituted of blood, fibrin and villi with chorionic cells.

Most of the villi show a normal stroma covered by a double layer of cells, the internal or Langhans and the external of syncytial. Many villi show projecting buds of proliferating cells.

A few chorionic cells are scattered in groups. The study of the relations between fetal and maternal tissue would greatly aid in throwing some light on the unsettled questions connected with ovarian pregnancy, and indirectly on the whole subject of ectopic gestation.

Decidual formation and embedding of the ovum in the ovary are still unsolved problems, owing to the infrequent occurrence of these cases, and absence of early uninterrupted specimens.

After the most careful study of all the sections I found what I would only call an attempt at decidual formation. Sparsely grouped cells are found along the margin of the ovum, or better in the boundary between fetal structure and ovarian stroma.

The cells are large polygonal, or oval, with one or two round nuclei, they took the stain very lightly and are embedded in a layer of fibrin which lies at the boundary of the two structures. Cells of similar characters are scattered in the ovarian stroma and around blood-vessels.

The fact is worth considering that no similar cells are found in the fibrin and fibrous tissue covering the inner aspect of the corpus luteum, or in the fibrous tissue constituting the septa of the corpus luteum.

This so-called attempt at decidual formation is more marked where the invasion of fetal elements into the ovarian stroma seems most active.

The shape, the size, and the manner of assuming the stain seem to differentiate them from the Langhans and syncytial cells; these last two varieties however seem to proceed into the tissue more closely grouped.

Whether the fibrinous layer surrounding the ovum and containing these cells is the representative of a true decidua is only a matter of conjecture. The alteration in the relations of tissue, due to the rupture, and changes due to delayed operation, is responsible for this unfavorable condition.

The principal features of this attempt at decidual formation, viz.:

(*a*) Its occurrence in the stroma of the ovary,

(*b*) Its marked appearance where the invasion of chorionic elements is more active,

(*c*) Its absence in proximity of the corpus luteum and fibrous constituents, are conditions which demand an explanation.

The first fact would indicate the reaction of a connective tissue to the specific action of the ovum. The connective tissue of the ovary would respond to the specific stimulus (invasive properties of the chorionic elements) with an attempt to resist the invasion. It is only logical to admit that the line of defense should be more marked where the invasion is greater.

The third condition is dependent on the unsettled questions connected with the formation and function of the corpus luteum.

The origin which the most recent investigators ascribe to the lutein cells from the connective cells of the internal theca, the apparent affinity of the decidual and lutein cells, the function ascribed to the corpus luteum of directing the normal development of the ovum, and controlling the invasive power of the chorionic elements, could possibly explain why the follicle should be considered good ground for the embedding of the ovum, and the synchronous formation of the corpus luteum should offer a relative resistance to the invasion of chorionic elements. In other words, it seems possible for the layer of lutein cells to take the place of the decidua in its functions.

Further, the absence of decidual cells in and about the corpus luteum, and the absence of chorionic cells outside of the corpus luteum, in the corresponding ovarian stroma, would seemingly substantiate such hypothesis.

But we need positive evidence, based on extensive experience, before we may arrive at such a conclusion. I wish here to emphasize

the fact of the intimate relation existing between the fetal tissue and the well-formed corpus luteum in my case, considering that only a thin fibrous layer connects the two structures. The most complete evidence of this fact is found in a careful study of the different sections in which it is easy to discern the progressive stages on the relation of the two structures. Some sections show the fetal elements completely enclosed within the corpus luteum, the latter forming a complete ring.

Others show the fetal tissue breaking through the lutein partitions, to pass directly outside into the ovarian stroma.

Still others present the appearance of the ovum almost completely crowding out the corpus luteum with only a small portion of the lutein cells remaining. This evidence seems to be conclusive for the implantation of the ovum directly in the follicle.

Microscopic Findings of Tubal Sections.—The sections taken from the ampullar part of the tube, and stained with hematoxylin-eosin, distinctly show the evidence of inflammatory processes.

The canal is smaller than ordinarily appears in the ampulla.

The wall is considerably thickened. The mucous membrane is not so arborescent as usual. The mucous folds are greatly reduced. The epithelium in some parts is flattened, proliferating and desquamating.

There is a very slight glandular appearance in the mucous folds, none in the stroma. The mucous stroma shows marked round-cell infiltration. This is present between the muscular bundles.

The external layer is considerably thickened, due to extensive proliferation of connective tissue. Edema of subperitoneal layer and considerable round-cell infiltration are present. There is no evidence of fetal elements anywhere in the tube.

CONCLUSIONS.

First, macroscopical and microscopical evidence establish beyond any doubt the primary ovarian nature of the gestation.

Second, the intimate relation of fetal tissue and corpus luteum in the various stages, might be a proof of the embedding of the ovum in the follicle.

Third, probably the formation of the corpus luteum partly assumes the function of the decidua only as a preventive of the invasion of the fetal elements. This view is in marked contrast with the views expressed by Teacher and Bryce, and the behavior of decidua and chorionic elements in their interlacing in the implantation site.

Fourth, decidual formation is only present as an incomplete attempt in the ovarian pregnancy, and is a result of the reaction of

the stroma to the specific action of the ovum. Every connective tissue in the genital organs is probably able to react in the same way as the Müllerian to the genetic influence.

Fifth, the incomplete formation of decidua explains the tremendous invasion of the stroma and its surroundings with chorionic elementi.

Sixth, this last condition is the cause of early interruption of ovarian pregnancy.

THE FECAL FLORA OF TYPHOID FEVER AND ITS REACTION TO VARIOUS DIETS

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THE FECAL FLORA OF TYPHOID FEVER AND ITS REACTION TO VARIOUS DIETS

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Altho the typhoid bacillus itself has been the subject of many investigations and much is known in regard to its biology and its pathogenesis, little exact information has been acquired in reference to the intestinal bacterial conditions which may be associated with an invasion by this bacillus. To be sure, typhoid fever is no longer to be regarded as essentially an intestinal disease, yet bacterial conditions in the intestines may be conceived to be such, in certain cases, as to add the weight of the toxic products of putrefaction to the human organism already oppressed by the typhoidal virus; in other patients, the fecal flora may be of a type which permits the most favorable digestion and absorption of the food administered.

As is well known, Coleman,[1] for a number of years, has been placing his typhoid patients on a high calory diet in order to reduce to a minimum the severe loss of nitrogen and weight which usually occurs in such cases when kept on a low calory diet. In connection with these cases in addition to careful clinical histories, a very accurate record is kept of the amounts of carbohydrate, fat, and protein consumed daily by each patient. The conditions are, accordingly, such as to present a most favorable opportunity to study the dietetic response of the typhoidal intestinal flora, and through the courtesy and cooperation of Dr. Warren Coleman the material for this study has been available.

As far as I am aware, this investigation is the first in which an attempt has been made to correlate conditions and changes in the fecal flora of human subjects with exact data in regard to the constitution of the diet utilized. It seems not unlikely that information of this character may give a clue to the most favorable dietaries for various infections, especially those involving the digestive tract. As Herter[2] has well suggested in connection with a general discussion of intestinal

1. Schaffer and Coleman: Arch. Int. Med., 1909, 4, p. 538.
2. Bacterial Infections of the Digestive Tract, New York, 1908, p. 181.

infections, "Our attention has, perhaps, been too exclusively fixed on the specific excitants, and the rôle played by associated bacteria must receive more study for it is clear that they sometimes play a significant part in determining the outcome of an infection. The difference that decides whether a man will live or die must frequently be a slight one looked at from the standpoint of the processes of battle within the body."

It has been repeatedly observed that a diet containing large amounts of carbohydrate not only encourages the development of a fermentative flora, but also, as Kendall[3] has recently pointed out, tends to protect the protein in the food from putrefactive decomposition, not alone by the obligate putrefactive bacteria, but also by members of the colon and proteus groups capable of exercising either putrefactive or fermentative activities. Altho much laboratory evidence has been advanced in support of this principle, little definite information has been adduced in regard to the amount of carbohydrate which should be incorporated in the diet of adults in order to bring about this result or in regard to the uniformity and degree to which this is effected in individuals with various types of fecal flora.

In this study, the aim has been to determine the biological propensities and activities of the fecal flora as a whole, rather than to isolate and classify individual bacteria which may happen to be present in the stools and in regard to numbers and significance of which there is no definite criterion. In order to effect this purpose, the methods introduced by Herter[4] and elaborated by Herter and Kendall,[5] in their feeding experiments with monkeys, and by MacNeal, Latzer, and Kerr,[6] in a study of the normal intestinal flora of man, have been adopted with some modifications. Altho the determination of the general type of flora has been the primary objective, the identification of bacterial types has always been effected when it would seem to serve a useful purpose.

This investigation was carried out during a part of the years 1912-1913.

During this period, a total of over one hundred stools from twenty-two typhoid patients have been examined. In a few instances, only a single specimen from a patient was subjected to the various procedures, but in twelve

3. Jour. Med. Research, 1911, 25, p. 117; Kendall and Farmer: Jour. Biol. Chem., 1912-13, 13, p. 63.
4. Bacterial Infections of the Digestive Tract, New York, 1907, p. 181.
5. Jour. Biol. Chem., 1909-10, 7, p. 203.
6. Jour. Infect. Dis., 1909, 6, pp. 123, 571.

cases a series of four to seventeen stools were investigated. With a few exceptions, these patients were from the metabolism ward of the second medical division of Bellevue Hospital. After October 1, 1913, the Russell Sage Institute of Pathology was in charge of this ward. It is a pleasure to acknowledge the assistance of Dr. Eugene F. DuBois and others of the Russell Sage Institute. I also wish to thank Dr. N. M. Keith of the New York Hospital for his aid.

METHODS

The material collected for examination was generally a part of a movement occurring between 6 and 7 o'clock in the morning. The stool was received in a sterile bed pan and a part transferred to a sterile bottle. These specimens were kept on ice during the few hours which might elapse before examination. As a number of the patients were inclined to constipation, it was frequently necessary to use an enema, consisting generally of a sterile normal saline solution.

With all the specimens, except those from fluid stools, a definite weighed amount of feces was used in seeding the various culture media. In making the dilutions of the feces, a method similar to that of MacNeal[6] was followed, viz., 500 mg. of fecal matter, representative of the whole specimen as regards moisture and consistency, were carefully weighed in a large sterile watch glass. This material was then thoroughly emulsified in 50 c.c. of normal salt solution and poured into a small flask. Each cubic centimeter, accordingly, held the equivalent of 10 mg. of the fecal matter. With fluid stools, an emulsion was made to the same density as one of these weighed preparations. From this primary emulsion, smears were made and treated with gram stain for microscopical examination and differential count.

A series of dilutions of the fecal emulsion at 1-10, 1-100, 1,-1,000, and 1-10,000 were next prepared and from suitable dilutions, using 1 c.c. in each case, poured plates were made with sugar-free agar, 1 percent lactose agar, and standard nutrient gelatin. These media were all titrated + 1.0 to phenolphthalein. The sugar-free agar plate was incubated aerobically and the lactose agar plate anaerobically at 37 C. Definite amounts of the diluted material were also spread on large Endo plates with sterile glass rods. The Endo medium was prepared in accordance with Kendall and Day's[7] modification. After twenty-four hours' incubation, typhoid-like colonies on the Endo plates were transferred to Russell's[8] double sugar medium and characteristic growths were subjected to agglutination and cultural tests.

The following media were seeded with 0.5 c.c. of the primary fecal emulsion: Fermentation tubes containing sugar-free meat infusion peptone broth plus 1 percent dextrose, lactose, and saccharose; a fermentation tube containing litmus milk; and tubes containing N/20, N/10, and N/5 acetic acid dextrose broth. This acetic acid medium was prepared in the following manner: Acetic acid was added to 1 percent dextrose meat infusion peptone broth until the reaction became N/5 acid to phenolphthalein. Part of this was then diluted with neutral dextrose broth until the N/10 and N/20 titrations were obtained. The three lots of this medium were tubed in 10 c.c. amounts and plugged with paraffined cotton. These tubes were incubated at 37 C.

The undiluted feces were used in seeding the following media: About 0.5 gm. was emulsified in the open arm of a large fermentation tube containing lactose peptone bile; a loop was streaked on a slant of Loeffler's serum medium;

7. Jour. Med. Research, 1911, 25, p. 95.
8. Ibid., p. 217.

and a stab culture was made in nutrient gelatin. The bile and serum media were incubated at 37 C. and the gelatin was incubated at room temperature.

About 10 c.c. of a 1-10 dilution of the original fecal emulsion were heated at 80 C. for fifteen minutes for spore cultures. One cubic centimeter was plated in sugar-free agar and in lactose agar, and also, in some instances, the same amount was added to fermentation tubes, containing milk with defibrinated rabbit or dog blood added, and to lactose blood-agar plates. These cultures were incubated at 37 C.

All the plate cultures containing lactose were incubated at 37 C. under anaerobic conditions. The method of Zinsser[9] was followed in making these anaerobic plate cultures and proved entirely satisfactory. To insure a higher degree of anaerobiosis and to protect the surfaces of the seeded plates from the pyrogallic sodium hydrate solution, the hardened inoculated medium was covered with a layer of sterile agar.

After twenty-four hours' incubation, the Endo plates, the sugar-free agar plates, and the fermentation tubes were examined and the results recorded. After forty-eight to seventy-two hours, the examination included the gelatin and the anaerobic plates, and also the acetic acid cultures. The spore cultures were incubated several days.

The gram stain, used in coloring the smears from the fecal emulsion and the sediments of the fermentation tubes, was prepared and employed as recommended by Elser and Huntoon.[10] The staining procedures were similar in each instance, except that the period of decolorization with absolute alcohol was varied in accordance with the thickness of the smear. In a number of instances, the results with this method were compared with results from the use of preparations decolorized with xylol and anilin oil followed by methyl alcohol, as recommended by MacNeal for work of this character. Sharper differentiation, however, was obtained by the treatment with absolute alcohol. A total of 300 bacteria was counted from each specimen and the results expressed in percentages. I am indebted to Mr. A. H. Rahe, my assistant, for many differential bacterial counts.

SIGNIFICANCE OF THE VARIOUS TESTS

The several procedures followed in these examinations were selected because, taken as a whole, they seemed to give a composite picture of the character of the stool and, through them, changes in the fecal flora following modifications in the diet could be satisfactorily followed. The general purpose of each test is as follows:

Endo Plates.—The primary object in the use of these plates was to gain an insight into the general character of the viable aerobic flora of the stool. With a little experience, it is possible from a mere inspection of these plates to identify a considerable number of different types of bacterial colonies, such as B. coli, B. lactis aerogenes, B. alkaligenes, the colon types which do not act on lactose and which for convenience are designated B. paracoli in the tables, also B. pyocyaneus, B. proteus, streptococcus, and staphylococcus. In the tables, the relative number of colonies appearing on these plates is indicated by the number of

9. Jour. Exper. Med., 1906, 8, p. 542.
10. Jour. Med. Research, 1909, 20, p. 377.

crosses, and the most numerous types of bacterial colonies are written below in order of prevalence. A search was always made for typhoid colonies and, as two or more plates with a favorable distribution of colonies were almost always obtained, viz., 250-300 colonies on a plate, the failure to find any typhoid colonies indicated that these bacilli were present in the feces in small numbers, if at all. Ficker and Hoffmann[11] estimated that, with the Drigalski plate medium, the isolation of typhoid bacilli from stools was unlikely in cases where the viable fecal bacteria outnumbered the typhoid bacilli more than 300 to 1. In this series of patients on the high calory diet, the typhoid bacillus was isolated from the stools infrequently either from directly seeded Endo plates or from bile enrichment tubes.

Fig. 1.—Lactose agar anaerobic plating, showing the grouping of the [illegible] colony about a central colon colony. X1.

Sugar-Free Agar Aerobic Plates and Lactose Agar Anaerobic Plates.—A comparison of these two culture plates has been found to give a very satisfactory index of the relative numbers of B. acidophilus which may be present in the stool. The various types of the colon group and the streptococci develop on both media, but the bacillus acidophilus and, at times, the bacillus bifidus appear only on the anaerobic lactose plates. The bacillus acidophilus develops very feebly, if at all, on media without sugar, and altho it will grow aerobically a higher degree of development occurs under anaerobic conditions. Such anaerobic lactose plates, seeded from stools containing many bacilli of

11. Arch. f. Hyg., 1904, 49, p. 229.

the acidophilus type, exhibit a peculiar and characteristic grouping of the colonies. At suitable dilutions, the colonies are seen to be grouped in clusters. In the center, there is at least one large lenticular colony which may or may not have an associated bubble of gas. Clustered about these central colon bacillus colonies are smaller colonies, often in great numbers, which decrease in size with increasing distance from the central colony until, at the periphery, they become exceedingly minute. In the neighborhood of these colony groups, the medium is clouded. The smaller colonies are composed of the bacillus acidophilus or, at times, of the bacillus bifidus and it is evident that their development is initiated and favored by the acid and possibly other growth

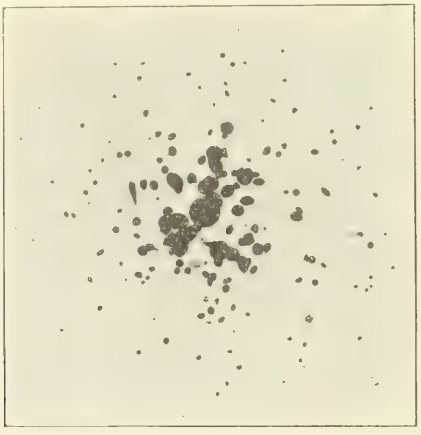

Fig. 2.—Detail of one of the clusters. X4.

products formed by the colon bacilli diffusing through the medium. These bacilli are evidently acidophilic as well as aciduric.[12] In some instances, the large central colonies were found to be composed of aciduric bacilli. In the culture plates containing several hundred colon bacilli the development of these acidophilic bacteria may be uniform throughout the plate. The difference in the count on the anaerobic lactose agar plates, exhibiting this peculiar grouping of the colonies and clouding of the medium, and that on the sugar-free agar plates or gelatin plates may be considered a fair index of the number of viable acidophilic bacilli in the stool. In the tables directly under the count

12. Kendall has suggested this name for the whole group of acid-tolerating bacteria, Jour. Med. Research, 1910, 22, p. 153.

for the anaerobic lactose agar plates, the type of the predominating microorganism is stated.

Acetic Acid Glucose Broth.—Additional information in regard to the acidophilic flora is gained from an inspection of the tubes containing three strengths of acetic acid, each of which has been seeded with 5 mg. of the feces. Aside from yeasts, the bacillus acidophilus was the only organism found in these stools capable of multiplying in this acid medium. While the lactose agar anaerobic plates reveal the number of B. acidophilus in the stool, the acid fluid media indicate especially the aciduric properties of these bacilli. Organisms which are capable of multiplying in a medium with an acidity of N/10, or even N/5, acetic acid have acquired marked acid resisting properties. An examination of stools from normal individuals, and also from those with a somewhat disturbed digestion, has shown that such highly aciduric bacilli are only rarely present in the intestinal tract. As a result, however, of a continued diet containing large amounts of carbohydrate associated with reduced amounts of protein and fat, such bacilli may be developed (Table 1). Their presence in the stool justifies the assumption that unusual amounts of lactic acid are being produced in the intestinal tract, especially in the region of the large intestine, which is the locality where these bacilli undergo their greatest development. It may also be assumed that carbohydrate is available in this locality for their metabolism.

Fermentation Tubes.—Herter and his associates have laid much stress on the value of fermentation tubes containing sugar broths as an aid to the bacterial analysis of the feces. Because of the transition from almost complete anaerobiosis to aerobiosis which such tubes offer, they believed that the bacterial development in the tubes represents closely the viable organisms originally present in the feces. Furthermore, certain bacteria, such as the bacillus bifidus and the bacillus aerogenes capsulatus, altho present in such small numbers in the stool as to be overlooked, may be brought to strong development in these tubes. They asserted also that the volume of gas produced in tubes seeded with the feces is of significance, being much reduced from the normal 25-30 percent in certain intestinal disorders. Too much stress, however, should not be laid upon the volume of gas, as MacNeal, Latzer, and Kerr encountered a great variation in the amount produced in the case of twelve supposedly normal individuals exam-

ined at frequent intervals for an extended period. Kendall[13] has found that, under normal conditions of the digestive system, closely similar amounts of gas are formed in the dextrose, lactose, and saccharose tubes.

In the tables, the amounts of gas formed in twenty-four hours are expressed in percentages. As the length of the closed arm of the fermentation tubes varied considerably, the figures given have greater comparative value than those obtained by direct measurement in millimeters.

In these typhoid cases, there was no definite deviation from the normal average in the amount of gas produced. The stools of patients on the high calory diet in the earlier stages of the infection, and also patients on a milk diet (Table 5), showed, for the most part, a somewhat greater production of gas with dextrose than with the other two sugars, whereas in the later periods generally after a rather prolonged feeding with lactose, the maximum amount of gas was formed in lactose. As has been observed by others, stools containing an abnormal proportion of streptococci give rise to a much reduced amount of gas, especially in the saccharose tube. In the cases of John T. (Table 1) and Howard F. (Table 3), this was regularly the case as regards saccharose. On the other hand, with increasing numbers of the bacillus acidophilus in the stools, the productions of gas in the dextrose and lactose tubes remained about equal. The largest amounts of gas did not occur during periods when the patients were fed large amounts of carbohydrates, as might be expected, but were encountered when the amount of fat in the diet was increased, and especially when the proportions of fat and protein to the carbohydrate were raised (Table 1, Enrico P.; Table 2, Christian M.; Table 5, Joseph P. and Torello D.).

A much larger amount of gas was regularly formed in the lactose bile fermentation tube than in the lactose broth tube, which was due, in part, to the heavier seeding of the former. These bile tubes offered an enrichment medium for the typhoid bacillus, which was of doubtful value; they were chiefly of service in permitting the development of the bacillus aerogenes capsulatus and other spore-bearing anaerobes of a more or less putrefactive type. An examination of the sediments from these tubes threw some light on the question of the fermentative or putrefactive nature of the feces with which they were seeded. Accordingly, these sediments have been classified in the tables in

13. Jour. Biol. Chem., 1909-10, 6, p. 257.

reference to their probable potential activity. Thus, smears from sediments showing a majority of the organisms of the bacillus acidophilus type are designated fermentative; those in which the larger numbers of the bacteria are of the colon and streptococcic types are termed peptolytic; while smears in which there were many bacilli of the aerogenes capsulatus type, together with other spore-bearing bacilli are classified as putrefactive.

Another medium regularly employed in fermentation tubes was litmus milk. Stools, in which the bacillus acidophilus is predominant, generally form a rather soft clot with little or no gas; with many streptococci, the clot is firmer and there is little gas; with the ordinary stool of the adult the clot is solid and shrunken, whey is expressed from the clot and there is formed 20-50 percent of gas. A production of 100 percent gas generally, but not always, means the presence of large numbers of B. welchii. The principal object in using these milk fermentation tubes was to detect the presence of bacteria able to digest casein under anaerobic conditions. For this purpose, the tubes were kept under observation for a period of about ten days. This occurred only rarely and in a moderate degree.

Loeffler Serum Medium Slant and Gelatin Stab Culture.—These media were inoculated with the undiluted feces in order to gain an idea of the relative numbers of bacteria in the various stools capable of digesting proteins of this character. The aerobic liquefying bacteria are supposed to develop especially in the small intestine. Herter and Kendall[5] determined, through feeding experiments, that the fecal flora of monkeys, placed on a high protein diet, caused a rapid liquefaction of these media, whereas when the monkeys were given a diet consisting largely of carbohydrate slight or no digestion occurred. With typhoid patients exhibiting a fermentative flora, there occurred slight or no digestion of serum medium, whereas the liquefaction of the gelatin medium was often marked.

Spore-Bearing Bacteria.—As has already been mentioned a suspension of the feces to the amount of 1 mg. per cubic centimeter was heated at 80 C. for fifteen minutes. One cubic centimeter of this heated suspension was plated in sugar-free agar and incubated aerobically at 37 C.; 1 c.c. in lactose agar and incubated aerobically at 37 C.; and in some instances, 1 c.c. was plated anaerobically in lactose agar to which 1 c.c. of defibrinated blood had been added and also

seeded in blood milk fermentation tubes, both being incubated at 37 C. The last two media favored especially the development of B. welchii. Comparative results indicated that about ten times as many spores of this type would develop in the blood lactose agar plates as in the same media without blood. When these spores were present in small numbers in the stools, under 40 per milligram, the characteristic growth reaction with disruption and partial digestion of the curd might be delayed for forty-eight to seventy-two hours.

MacNeal found in normal human stools an average per milligram of 65 spores developing aerobically and 1,790 anaerobically. In these stools from typhoid cases on the high calory diet, I have rarely found as many spores as this; in fact, in many instances, no colonies at all developed on the plates seeded with the heated suspension of the feces. Those which did appear on the anaerobic plates were almost invariably of the bacillus welchii type. Their occurrence in relation to the diet will be discussed in the following section.

Direct Smears from the Feces Stained with Gram's Method.—In the original tabulations of the bacteria observed in the gram-stained fecal smears, fourteen different types were enumerated. In compiling the tables, however, it has been necessary to condense this enumeration and to group the bacteria under a few general types, which may lay claim to some distinction in morphology or staining, and which reacted definitely to changes in diet. The gram-negative bacilli were almost entirely of the colon type. The gram-negative cocci were present in such small numbers, if at all, as to be negligible. No spirochetes were observed. Within the aciduric bacilli group have been placed nearly all of the gram-positive rods without spores seen in the fecal smears. A study of pure cultures of aciduric bacilli isolated from these stools has shown that they may vary markedly in morphology. The most frequently encountered type, taken for the bacillus acidophilus, was a rather long, slender bacillus, about 3-6 microns, with rounded ends and strongly gram-positive. This bacillus, however, often occurs as a gram-positive, rather short, plump rod, 1-3 microns, and with rounded ends. There are also gram-positive, long, thick rods which belong in the aciduric group, but which cannot, from their morphology alone, be distinguished from the non-spore-bearing representatives of the bacillus aerogenes capsulatus. Certain gram-positive bacilli of the length of the first type of the bacillus acidophilus mentioned, but rather thicker, often curved and irregularly

staining, and with a punctate or bifid end, were not infrequently encountered. These were regarded as representatives of the bacillus bifidus and placed in the aciduric group. Logan[14] has recently directed attention to the marked polymorphism displayed by the various members of this acid-tolerant group.

The tables show a fairly close correspondence between the results obtained by the cultural procedures and the direct counts from the fecal smears. Of the two, however, the cultural tests give the more reliable information in regard to the actual bacterial conditions in the intestinal tract. This is due, in part, to the uncertainty of identification of the more important intestinal bacteria by morphology alone, and, in part, to the fact that an inspection of a gram-stained smear gives no information in regard to the viability of the bacteria. The number of viable bacteria in a stool in ratio to the total count has been estimated at anywhere from 1-100 (Klein) to 1-3,000 (MacNeal). Where there is a radical lack of correspondence in the tables between the cultural results and the direct count, it is probable that the discrepancy is due to an unusually large proportion of dead bacteria. This was especially apt to occur following marked changes in the diet.

In a number of cases, gram-stained smears from the sediments of the broth fermentation tubes after twenty-four hours' incubation were compared with direct counts from the stool. A comparison of sediments of the dextrose, lactose, and saccharose tubes, seeded from the same material, did not show any uniform difference in the character of the sediments, altho, in some instances, streptococci had multiplied more actively in the saccharose tube, and the bacillus bifidus appeared in larger numbers in the sediments from the dextrose and lactose tubes. The counts indicate, as Herter and Kendall[15] have claimed, that the growths in these fermentation tubes represent, in a measure, the bacteria originally present in the feces. They are not present however in the same relative numbers. Streptococci and also the bacillus acidophilus, if present in any considerable numbers in the stool, always multiplied to such a degree as to far outnumber the colon bacillus types in the sediments. The most interesting type sometimes brought to active development was the bacillus bifidus. This occurred at times in tubes seeded with feces in the smears of which the bacillus bifidus was seen in very small numbers, or not at all.

14. Jour. Path. and Bacteriol., 1914, 18, p. 527.
15. Jour. Biol. Chem., 1908-9, 5, p. 283.

It may be mentioned that in two watery stools with much mucus from two typhoid patients with active diarrhea the bacillus bifidus, including many bifid forms, was observed in the fecal smears in very large numbers, in one instance constituting 55 percent of the total bacteria, and in the other 29 percent. As this bifid bacillus has been encountered rarely in the stools of normal adults, its presence in such large numbers in these pathological adult stools is worthy of note.

DESCRIPTION OF THE TABLES

It was not feasible to attempt the detailed publication in a tabulated form of the findings in the total 103 stools examined. Accordingly, only a number of selected cases have been presented in such detail as space permitted. These, however, illustrate the salient features revealed in this work. In each of Tables 1-4 have been grouped two or more cases which illustrate the response of a certain type of fecal flora to various modifications of the high calory diet, while in Table 5 are given the results with a milk diet in two cases. In the following section, there is a discussion of the various types of flora encountered and their reactions to the different diets.

All of the counts from the plate cultures refer to the numbers of colonies appearing per milligram of the feces. The figures for the fermentation tubes indicate the percentage of gas formed. In indicating the degree of liquefaction of gelatin and Loeffler serum, the following symbols are used: ±, slight; +, moderate; + +, marked; + + +, rapid; + + + +, very rapid. As regards the amount of growth in the acetic acid glucose broth tubes: 1 means slight growth; +, moderate; + +, heavy; + + +, very heavy.

In Tables 1-4, the average daily amounts of proteins, fats, and carbohydrates given each patient are expressed in grams. Unless otherwise stated, the figures signify the average for the period since the last examination. In the first column, the diet for each patient since admission to the hospital is described.

The following brief case-reports include only such of the patients on the high calory diet as are incorporated in the tables.

John T.—Twenty-five years old, admitted November 19, 1912, on the eighth day of the disease. Onset five days ago with headache and vomiting. Since then the patient has had anorexia, malaise, and several nose bleeds. Looks fairly well nourished but weak and acutely ill.

On November 21, agglutination test positive and typhoid bacilli recovered from the blood; November 20, several rose spots appeared, small abdominal

hemorrhage in the evening; November 23, spleen palpable; November 25, abdomen slightly tympanitic, phlebitis in right thigh; November 26 to 29 and on December 1 patient had chills; December 4, general condition improved, patient less stuporous; December 31, temperature shot to 103, both femoral regions painful and swollen; January 1, temperature normal. Right leg remained swollen during convalescence. Patient gained rapidly in weight.

Enrico P.—Twenty-four years old, admitted December 4, 1912, on the fourth day of the disease. Two days ago had severe cough with blood-tinged expectoration. Appetite failed and patient suffered from headache. Looked fairly well nourished, but acutely ill; many large and small moist râles in lungs; spleen palpable.

December 5, agglutination test positive and typhoid bacilli recovered from the blood, patient apathetic, expectoration and headache continues, no rose spots; December 10, abdomen slightly distended, signs of bronchitis; December 14, spleen palpable 2 cm. below costal margin, general condition good, nutrition good; January 3, stools continue green and contain mucus, diet almost exclusively carbohydrate had not changed character of the stools, patient put on fat diet for three days, stools changed markedly, became light brown and formed, takes fat diet well; January 15, patient seems to have intolerance for lactose, whenever given 50 to 60 gm. shows distention, passes large amounts of gas and suffers considerable abdominal pain. Convalescence rapid. Patient kept for examination of feces.

Anthony G.—Eight years old, admitted August 19, 1913, on the eleventh day of the disease. About two weeks ago began to be apathetic, lost appetite, and suffered from headaches; in bed since August 15. Looked fairly well nourished and well developed; spleen not palpable; several rose spots; agglutination doubtful.

On August 23, severe abdominal pains, no sign of perforation, abdomen much distended; August 25, many rose spots, spleen palpable. No more abdominal distention. Convalescence rapid.

Christian M.—Thirty-one years old, admitted September 8, 1913, on the eleventh day of the disease. About two weeks ago began to have headaches and malaise, four days ago stopped work, next day developed diarrhea. Very well developed; apathetic.

On September 8, agglutination was positive and typhoid bacilli recovered from the blood, spleen not palpable, rose spots appeared in crops; September 19, pain and tenderness in right lumbar region lasting several days; October 4 to 15, typhoid bacilli in urine and feces, disappearing later.

Thomas B.—Sixty years old, admitted October 2, 1913, on the fifteenth day of the disease. For two weeks has suffered from malaise and anorexia; has been feverish. A large, well-developed man, looks acutely ill, apathetic.

On October 21, many rose spots, agglutination positive, blood culture positive for typhoid bacilli.

October 13, rose spots on abdomen and back, spleen not palpable; October 27, uninterrupted convalescence; November 10 to 26, serofibrinous pleurisy, fluid base of left lung.

Morris S.—Twenty-one years old, admitted August 17, 1913, on the seventh day of the disease. Seven days ago began to have pains in abdomen and back and since then loss of appetite and constipation. He has a short, small frame, well nourished; distinct pyorrhea alveolaris; spleen palpable.

On October 18, agglutination negative, blood culture positive for typhoid bacilli; October 22, complains of sore throat; October 23, condition good, slightly apathetic, spleen palpable 4 cm. below costal margin, abdomen slightly distended;

October 24, agglutination positive; October 27, abdomen which was distended on high carbohydrate and very low fat diet became soft when the amount of fat was increased; October 30, a little irrational, color grayish, pulse soft; November 4, general condition improved; November 17, temperature rising, patient feels well, does not look sick; November 18, sharp pains right side of abdomen lasting a few hours; November 24, patient not toxic in this relapse; December 3, feels well, patient had frothy stools on high carbohydrate, but when amount of fat increased became normal; December 17 to 25, second relapse, patient not at all sick, spleen not palpable, no rose spots. Throughout the long febrile period the patient's state of nourishment remained very good and convalescence was rapid.

Howard F.—Twelve years old, admitted November 4, 1913, on the fourth day of the disease. On October 26 severe headaches and malaise; October 29, developed chills and took to bed. His uncle, Charles F., living in the same house developed typhoid at the same time. Tall, well-developed boy; teeth good; good state of nutrition; spleen not palpable; several rose spots.

On November 8, condition good, rational but apathetic; November 12, signs of bronchitis; November 14, blood culture positive for typhoid bacilli; November 17, takes food poorly, much emaciated, somewhat toxic; December 10, passed two ascarides. Convalescence rather slow, heart somewhat enlarged, action rapid on exertion.

Charles F.—Twenty-four years old, admitted November 4, 1913, on the eighth day of the disease. About a week ago lost appetite and since then has suffered from headaches and malaise. Medium frame, well nourished, spleen palpable, several rose spots.

On November 5, agglutination positive; November 6, blood culture positive for typhoid bacilli; November 8, rational, slightly toxic, several small intestinal hemorrhages; November 19, intestinal hemorrhage of about 250 c.c., patient much prostrated, takes food badly, many rose spots in crops; November 14 to 17, developed severe follicular tonsillitis, became very toxic and apathetic, pulse of poor quality; December 3, condition much improved. Convalescence fairly rapid.

Thomas F. Thirty-four years old, admitted August 22, 1913, on the fourteenth day of the disease. About two weeks ago began to suffer from headaches and malaise. Well developed and well nourished; rough systolic murmur at base of heart; spleen not palpable.

On August 27, many rose spots; August 29, very apathetic; September 2, blood culture positive for typhoid bacillus, small abscesses on buttocks, has had much abdominal distention at times, irrational; September 5, abscesses incised; September 8, emaciation has been very rapid; September 12, showed much improvement. Convalescence rather slow.

Emil C.—Twenty-three years old, admitted August 23, 1913, on the fifth day of the disease. Five days ago suffered from fever, chills and headache; four days ago colicky pains in stomach, appetite good. Well nourished, does not look acutely ill; spleen palpable; doubtful rose spots.

On August 26, agglutination positive; August 29, blood culture positive for typhoid bacilli; September 1, more rose spots, severe headache; September 5, abdominal distention moderate; September 19 to 21, complains of headache; September 24, began to relapse; September 26, temperature rose to 104 F., spleen palpable 6 cm. below costal margin, several rose spots. During convalescence, October 18 to 30, temperature elevated, cause unknown.

TABLE 1

EFFECT OF HIGH CALORY DIET CONTAINING MUCH CARBOHYDRATE ON THE FECAL FLORA OF TWO TYPHOID PATIENTS WITH INITIAL FACULTATIVE TYPES OF FLORA

John T., Age 25, Admitted Nov. 19, 1912, on Eighth Day of Disease. Convalescent, Jan. 1, 1913. Moderately Severe Typhoid.

Date	Nov. 22	Nov. 25	Nov. 29	Dec. 6	Dec. 12	Dec. 19	Dec. 27	Jan. 15
Character of stool	Semi-fluid (much blood)	Fluid (no blood)	Semi-formed	Semi-formed	Semi-formed	Formed	Formed	Formed
Endo plate	++ B. coli B. typhosus Streptococcus	++++ Streptococcus B. coli	+++++ Streptococcus B. coli	++ B. coli Streptococcus	++ B. coli Streptococcus	+ B. coli	++++ B. coli	++ B. coli Streptococcus B. aerogenes
Typhoid bacillus	+	—	—	—	—	—	—	—
Lactose agar plate, anaerobic, 37 C.	140,000 B. coli	750,000 Streptococci	3,000,000 Streptococci	1,750,000 B. acidophilus	500,000 B. acidophilus	240,000 B. acidophilus	1,100,000 B. acidophilus	40,000 B. acidophilus
Gelatin plate, aerobic, 21 C.	110,000	1,000,000 Streptococcus	450,000	8,000	3,500	1,500	500,000	32,000
Sugar broth 5% dextrose	20	2	5	25	35	22	32	25
Sugar broth 5% lactose	5	5	10	20	20	25	35	40
Sugar broth 5% saccharose	10	0	2	10	15	28	28	15
Acetic acid glucose broth N/20	—	+	1	++	+++	++	++	+
Acetic acid glucose broth N/10	—	—	—	++	++	++	++	1
Acetic acid glucose broth N/5	—	—	—	+	++	+	+	—
Smear: Total gram-positive bacteria	11.7	8.8	75.7	100.0	94.0	92.0	84.6	75.3
Smear: Total gram-negative bacilli	5.3	2.0	24.3	.0	6.0	8.0	15.4	24.7
Smear: Total aerobic bacilli	28.3	10.6	16.2	97.1	81.9	88.2	69.2	71.9
Smear: Total streptococci	9.9	53.4	87.6	2.6	4.0	7.6	3.6	.6
Smear: Total staphylococci	19	.9	1.6	.0	.0	.0	2.3	.0
Diet: Protein grams	Milk 3000	58.5	75.0	120.0	121.5	120.5	120.5	99.5
Diet: Fat grams	2000 c.c.	75.5	103.0	140.6	178.2	198.2	205.0	166.0
Diet: Carbohydrates grams		325.6	381.3	293.6	406.8	447.5	451.2	409.5
Temperature	103	99.5	100	100.2	101.2	100	99.5	98.4

TABLE 1—(*Continued*)

Enrico P., Age 24, Admitted Dec. 4, 1914, on Fourth Day of Disease. Convalescent, Feb. 11, 1914. Moderately Severe Typhoid.

Date	Dec. 7	Dec. 14	Dec. 28	Jan. 10	Feb. 6	Feb. 11	Feb. 21	Feb. 26
Character of stool	Semi-formed	Pultaceous	Semi-formed	Formed	Semi-formed	Semi-formed	Formed (moist)	Semi-formed
Endo plate	++ B. coli	++++ B. coli	++ B. coli B. aerogenes	+++++ Streptococcus B. coli B. aerogenes	++ B. coli B. aerogenes	1 Streptococcus B. coli	++++ B. coli	+ B. coli B. aerogenes
Typhoid bacillus	—	—	—	—	—	—	—	—
Lactose agar plate, anaerobic, 37 C.	260,000 B. coli	4,200,000 Streptococci	450,000 B. acidophilus	2,000,000 B. acidophilus	1,000,000 B. acidophilus	200,000 B. acidophilus	500,000 B. coli	3,300 B. coli
Gelatin plate, aerobic, 21 C.	520,000	7,600,000	600	31,000	5,000	900	370,000	2,400
Sugar broth fermentation: Dextrose	40	—	25	28	25	30	45	60
Sugar broth fermentation: Lactose	35	30	28	28	30	28	55	52
Sugar broth fermentation: Saccharose	25	10	25	25	33	25	40	48
Acetic acid glucose broth N/20	+	++	++	++	+	+	+	1
Acetic acid glucose broth N/10	—	+	+	+	1	+	+	—
Acetic acid glucose broth N/5	—	—	—	1	—	—	—	—
Smear from stool: Total gram-positive bacteria	58.9	46.6	100.0	97.0	84.3	86.7	5.6	42.5
Smear from stool: Total gram-negative bacilli	41.1	53.4	.0	3.0	15.7	13.7	94.4	57.5
Smear from stool: Total aciduric bacilli	27.9	38.3	99.2	95.3	57.6	84.2	4.0	34.3
Smear from stool: Total streptococci	6.6	1.0	.3	1.6	2.6	2.3	.3	2.0
Smear from stool: Total spores	.3	.0	.0	.0	.0	.0	1.3	2.6
Diet in grams: Protein	Milk diet	73.0	79.2	65.1*	110.4†	144.5	131.8	111.1
Diet in grams: Fat	for 4 days	94.8	124.2	171.7	121.1	153.0	158.0	149.6
Diet in grams: Carbohydrate	.....	240.3	371.6	259.0	284.1	328.4	146.6	123.3
Temperature	103.5	104	102	99.5	98.4	98.0	98.4	98.4

* Average past 7 days.

† Average past 6 days.

TABLE 2

EFFECT OF HIGH CALORY DIET ON INTESTINAL BACTERIA OF TYPHOID PATIENTS WITH AN INITIAL FERMENTATIVE TYPE OF FLORA

Anthony G., Age 18, Admitted Aug. 19, 1913, on Eleventh Day of Disease. Convalescent, Sept. 8, 1913. Mild Typhoid.

Date	Aug. 22	Aug. 26	Sept. 2	Sept. 8
Character of stool	Pultaceous	Pultaceous	Pultaceous	Semi-formed
Endo plate	+++ Streptococcus B. coli B. aerogenes	++ B. coli B. aerogenes Streptococcus B. typhosus	++++ B. coli B. aerogenes Streptococcus	++ B. coli Streptococcus B. aerogenes
Typhoid bacillus	−	+	−	−
Sugar-free agar plate, aerobic, 37 C.	225,000	100,000	850,000	100,000
Lactose agar plate, anaerobic, 37 C.	1,450,000 B. acidophilus	1,400,000 B. acidophilus	1,300,000 B. acidophilus	1,250,000 B. acidophilus
Spores, aerobic, 37 C.	1	0	1	0
Spores, anaerobic, 37 C.	0	0	0	0
Lactose bile fermentation tube: Gas	40	30	50	45
Lactose bile fermentation tube: Sediment	Peptolytic	Fermentative	Fermentative	Fermentative
Gelatin stab, 21 C.	+	−	+	−
Loeffler serum slant, 37 C.	++	±	±	±
Sugar broth fermentation: Dextrose	30	25	38	40
Sugar broth fermentation: Lactose	29	28	25	45
Sugar broth fermentation: Saccharose	28	18	25	15
Acetic acid glucose broth N/20	++	+	+	+
Acetic acid glucose broth N/10	−	+	+	+
Acetic acid glucose broth N/5	−	−	−	−
Smear from stool: Total gram-positive bacteria	66.0		100.0	92.0
Smear from stool: Total gram-negative bacilli	34.0		0.0	8.0
Smear from stool: Total aciduric bacilli	62.2		98.6	85.1
Smear from stool: Total streptococci	0.3		0.6	1.0
Smear from stool: Total spores	0.0		0.0	0.0
Diet in grams: Protein	Milk diet	44.2	86.8*	58.1
Diet in grams: Fat	since	57.6	177.1	221.1
Diet in grams: Carbohydrate	Aug. 19	149.9	200.1	224.1
Temperature	103.2	101.6	99.6	98.4

* Average for 2 days.

TABLE 2.—(*Continued*)

Christian M., Age 31, Admitted Sept. 8, 1913, on Eleventh Day of Disease. Convalescent, Sept. 23, 1913. Mild Typhoid.

Date	Sept. 17	Sept. 22	Sept. 27	Sept. 30
Character of stool	Pultaceous	Formed	Formed (rather dry)	Pultaceous
Endo plate	++++ B. coli Streptococcus B. aerogenes	++++ B. coli Streptococcus B. aerogenes	+++ Streptococcus B. coli Staphylococcus	+++++ Streptococcus B. coli B. paracoli
Typhoid bacillus	—	—	—	—
Sugar-free agar plate, aerobic, 37 C.	520 000	325,000	56,000	1,100,000
Lactose agar plate, anaerobic, 37 C.	2,730,000 B. acidophilus	3,450,000 B. acidophilus	50,000 B. coli	4,100,000 B. acidophilus
Spores, aerobic, 37 C.	0	0	7	0
Spores, anaerobic, 37 C.	0	0	0	0
Lactose bile fermentation tube: Gas	33	65	75	48
Lactose bile fermentation tube: Sediment	Fermentative	Fermentative	Peptolytic	Fermentative
Gelatin stab, 21 C.	+	++	+	+++
Loeffler serum slant, 37 C.	±	±	++	++
Sugar broth fermentation: Dextrose	18	18	35	12
Sugar broth fermentation: Lactose	20	33	45	18
Sugar broth fermentation: Saccharose	20	10	18	10
Acetic acid glucose broth N/20	++	+++	1	+++
Acetic acid glucose broth N/10	+	++	—	++
Acetic acid glucose broth N/5	—	1	—	—
Smear from stool: Total gram-positive bacteria	52.0	55.3	86.6	36.3
Smear from stool: Total gram-negative bacilli	48.0	44.7	14.4	63.6
Smear from stool: Total aciduric bacilli	48.6	45.9	63.2	30.9
Smear from stool: Total streptococci	1.3	1.3	4.0	2.6
Smear from stool: Total spores	0.0	0.0	0.0	0.0
Diet in grams: Protein	80.5*	94.1	96.8	111.8
Diet in grams: Fat	122.1	74.2	206.5	155.9
Diet in grams: Carbohydrate	349.6	483.4	209.8	457.6
Temperature	101.8	99.4	99.0	99.5

* Average for 3 days.

TABLE 2.—(*Continued*)

Morris S., Age 25, Admitted Oct. 17, 1913, Seventh Day of Disease. Relapsed Nov. 15-Dec. 5, 1913; relapsed Dec. 17-Dec. 25, 1913. Severe Typhoid.

Date	Oct. 24	Oct. 28	Nov. 6	Nov. 11	Nov. 21
Character of stool	Fluid	Pultaceous	Semi-formed	Pultaceous (frothy)	Fluid
Endo plate	+ B. coli Streptococcus	— Streptococcus B. coli B. paracoli	— B. coli	+ B. coli	++ B. aerogenes B. coli Streptococcus
Typhoid bacillus	—	—	—	—	—
Sugar-free agar plate, aerobic, 37 C.	16,000	100,000	520,000	48,000	2,000,000
Lactose agar plate, anaerobic, 37 C.	1,200,000 B. acidophilus	1,800,000 B. acidophilus	6,800,000 B. acidophilus	1,900,000 B. acidophilus	1,170,000 B. acidophilus
Spores, aerobic, 37 C.	0	5	0	0	3
Spores, anaerobic, 37 C.	0	0	2	0	1
Lactose bile fermentation tube: Gas	66	75	75	75	75
Lactose bile fermentation tube: Sediment	Fermentative	Fermentative	Fermentative	Fermentative	Fermentative
Gelatin stab, 21 C.	±	+	±	±	±
Loeffler serum slant, 37 C.	+++	++	++	+	±
Sugar broth fermentation: Dextrose	20	65	20	16	8
Sugar broth fermentation: Lactose	18	32	30	25	9
Sugar broth fermentation: Saccharose	2	10	5	8	3
Acetic acid glucose broth N/20	+++	+++	+++	—	—
Acetic acid glucose broth N/10	+++	++	+++	—	+
Acetic acid glucose broth N/5	±	+	±	—	±
Smear from stool: Total gram-positive bacteria	74.3	85.7	38.4	88.0	81.7
Smear from stool: Total gram-negative bacilli	25.7	14.3	12.6	12.0	18.3
Smear from stool: Total aciduric bacilli	64.1	75.6	52.2	85.6	84.2
Smear from stool: Total streptococci	2.6	6.0	1.6	0.0	0.6
Smear from stool: Total spores	0.0	0.0 (Many yeasts)	0.0	0.0 (Many yeasts)	0.6
Diet in grams: Protein	Oct. 21-24. 2,000-3,000 calories with high carbohydrate	102.8	95.1	99.4	98.6
Diet in grams: Fat		80.9	128.2	74.3	109.6
Diet in grams: Carbohydrate		374.9	294.3	140.1	365.1
Temperature	103	103.5	100	99.4	100

* See Page 34.

TABLE 3

EFFECT OF MODERATELY HIGH CALORY DIET ON THE FECAL FLORA OF TWO SEVERE TYPHOID PATIENTS WITH AN INITIAL FACULTATIVE TYPE OF FLORA

Howard F., Age 12 Years, Admitted Nov. 4, 1913, on Fourth Day of Disease. Convalescent, Nov. 28, 1913. Severe Typhoid.

Date	Nov. 7	Nov. 11	Nov. 17	Nov. 21	Nov. 28	Dec. 10
Character of stool	Formed (moist)	Formed (moist)	Pultaceous	Semi-formed	Formed (moist)	Formed (moist)
Endo plate	+++ B. coli Streptococcus	+++ B. coli B. aerogenes Streptococcus	+++ Streptococcus B. coli	+++ B. coli Streptococcus	+++++ Streptococcus B. coli	+++ B. coli
Typhoid bacillus	−	+	−	−	−	−
Sugar-free agar plate, aerobic, 37 C.	175,000	96,000	300,000	545,000	1,130,000	480,000
Lactose agar plate, anaerobic, 37 C.	143,000 B. coli	170,000 B. coli	550,000 B. acidophilus	885 000 B. acidophilus	1,350,000 Streptococci	1,150,000 B. acidophilus
Spores: Aerobic, 37 C.	0	0	0	0	5	3
Spores: Anaerobic lactose agar, 37 C.	0	2	0	0	450	1
Spores: Anaerobic blood lactose agar, 37 C.	.....	.....	.....	38	10,000	34
Gelatin stab 21 C.	−	±	±	±	+	+
Loeffler serum slant, 37 C.	++	+++	+++	+++	+	+
Sugar broth fermentation: Dextrose	35	50	40	25	30	20
Sugar broth fermentation: Lactose	42	30	30	38	40	18
Sugar broth fermentation: Saccharose	5	18	10	10	5	2
Acetic acid glucose broth N/20	+++	++	+++	+++	+++	+++
Acetic acid glucose broth N/10	−	+	+++	++	++	++
Acetic acid glucose broth N/5	−	−	−	−	−	−
Smear from stool: Total gram-positive bacteria	57.3	60.0	93.6	93.3	13.3	54.0
Smear from stool: Total gram-negative bacilli	42.7	40.0	6.4	6.3	86.7	46.0
Smear from stool: Total aciduric bacilli	52.5	55.2	85.6	92.2	10.6	51.2
Smear from stool: Total streptococci	4.3	4.3	8.0	3.6	2.3	2.6
Smear from stool: Total spores	0.0	0.0	0.0 (Many yeasts)	0.0	0.0	0.0
Diet in grams: Protein	89.9	46.6	36.7	43.3	68.2	99.5
Diet in grams: Fat	80.8	79.0	46.8	71.4	119.5	120.4
Diet in grams: Carbohydrate	42.1	91.8	101.4	97.8	132.4	352.0
Temperature	102.2	104	102.6	103.2	98.4	99

TABLE 3—(*Continued*)

Charles F., Age 24 Years, Admitted Nov. 4, 1913, on Eighth Day of Disease. Convalescent, Dec. 2, 1913. Severe Typhoid.

Date	Nov. 7	Nov. 10	Nov. 17	Nov. 21	Nov. 28	Dec. 10
Character of stool	Formed (dry)	Pultaceous	Semi-formed	Fluid	Formed (rather dry)	Semi-formed
Endo plate	+ B. coli	+ B. coli B. paracoli	++++ Streptococcus B. coli	+++++ Streptococcus B. paracoli, B. coli	++ Streptococcus B. coli	++ Streptococcus B. coli
Typhoid bacillus	—	—	—	—	—	—
Sugar-free agar plate, aerobic, 37 C.	235,000	60,000	235,000	520,000	230,000	210,000
Lactose agar plate, anaerobic, 37 C.	175,000 B. coli	85,000 B. coli	340,000 B. coli	91,000 B. coli	365,000 B. acidophilus	365,000 Streptococci
Spores: Aerobic, 37 C.	0	....	5	1	11	46
Spores: Anaerobic lactose agar, 37 C.	0	.....	.....	0	5	0
Spores: Anaerobic blood lactose agar, 37 C.	....	.....	.....	40	2	4
Gelatin stab 21 C.	+	+ +	+	—	+	+ +
Loeffler serum slant, 37 C.	—	+	+ + +	±	+	+
Sugar broth fermentation: Dextrose	40	17	25	38	20	25
Sugar broth fermentation: Lactose	40	23	15	28	35	33
Sugar broth fermentation: Saccharose	15	5	8	15	20	37
Acetic acid glucose broth N/20	+ +	+	+	+ + +	+ + +	+ + +
Acetic acid glucose broth N/10	1	1	—	1	+ + +	1
Acetic acid glucose broth N/5	—	—	—	—	—	—
Stained: Total gram-positive bacteria	41.0	61.0	78.6	68.0	41.0	57.0
Stained: Total gram-negative bacilli	59.0	39.0	21.4	32.0	59.0	43.0
Stained: Total acid-fast bacilli	33.6	33.6	68.6	64.6	32.2	52.2
Stained: Total streptococci	5.6	5.6	5.3	5.0	3.0	2.3
Stained: Total spores	0.0	0.0 (Many yeasts)	0.0	0.0	0.0	0.0
Diet: Protein	Milk and whites of eggs	62.4	46.3	48.9	74.4	85.2
Diet: Fat		138.9	86.3	72.3	129.8	123.2
Diet: Carbohydrate		122.8	181.4	105.4	132.9	322.3
Temperature	104	103	105	104.6	103.4	99

TABLE 4

TYPHOID CASES WITH AN INITIAL PUTREFACTIVE FLORA

Thomas F., Age 34, Admitted Aug. 22, 1913, on Fourth Day of Disease. Convalescent, Oct. 2, 1913. Severe Typhoid.

Date	Aug. 26	Aug. 29	Sept. 4	Sept. 13	Sept. 19
Character of stool	Formed (moist)	Pultaceous	Semi-fluid	Pultaceous	Pultaceous
Endo plate	++ B. coli Streptococcus B. pyocyaneus	+++ B. coli Streptococcus B. aerogenes	+++ B. coli Streptococcus B. aerogenes	+++ B. coli Streptococcus B. typhosus	++ B. typhosus B. alkaligenes B. coli
Typhoid bacillus	—	—	—	+	+
Sugar-free agar plate, aerobic, 37 C.	160,000	730,000	290,000	700,000	130,000
Lactose agar plate, anaerobic, 37 C.	180,000 B. coli	1,450,000 Streptococci	215,000 B. coli	900,000 B. coli	162,000 B. acidophilus
Spores, aerobic, 37 C.	5	0	625	0	2
Spores, anaerobic, 37 C.	560	0	0	0	0
Lactose bile fermentation tube: Gas	60	18	42	35	50
Lactose bile fermentation tube: Sediment	Putrefactive	.....	Peptolytic	Peptolytic	Peptolytic
Gelatin stab, 21 C.	++++	±	+++	±	++
Loeffler serum medium, 37 C.	++++	++	+++	+++	++++
Sugar broth fermentation: Dextrose	25	35	20	30	48
Sugar broth fermentation: Lactose	30	25	35	28	30
Sugar broth fermentation: Saccharose	20	12	15	15	12
Acetic acid glucose broth N/20	—	+	+	+	+
Acetic acid glucose broth N/10	—	1	—	—	+
Acetic acid glucose broth N/5	—	—	—	—	—
Total gram-positive bacteria	16.3	69.3	49.3	59.3	27.6
Total gram-negative bacilli	83.7	30.7	50.7	40.7	72.4
Total aciduric bacilli	14.2	29.6	42.3	63.6	25.2
Total streptococci	1.3	7.6	2.3	2.6	2.6
Total spores	0.0	2.3	0.0	0.0	0.0
Diet in grams: Protein	Milk	48.4	50.1	84.4	94.8
Diet in grams: Fat	since	78.6	71.6	159.2	173.1
Diet in grams: Carbohydrate	Aug. 22	135.6	144.7	260.6	480.6
Temperature	105	104.5	103	100	100.5

TABLE 4.—(*Continued*)

James C., Age 52, Admitted Aug. 24, 1913, on Seventeenth Day of Disease. Died Aug. 29, 1913. Emil C., Age 23, Admitted Aug. 23, 1913, on Fifth Day of Disease. Convalescent, Oct. 17, 1913. Severe Typhoid.

Date	James C. Aug. 25	Emil C. Sept. 6	Sept. 16	Sept. 22	Oct. 6
Character of stool	Semi-fluid	Pultaceous	Formed (moist)	Pultaceous	Formed [illegible]
Endo plate	++ B. coli B. typhosus Streptococcus	++++ B. coli Streptococcus B. aerogenes	++ B. coli B. aerogenes Streptococcus	++++ B. coli Streptococcus B. aerogenes	++++ B. coli
Typhoid bacillus	+	−	−	−	+*
Sugar-free agar plate, aerobic, 37 C.	52,000	520,000	145,000	730,000	[illegible]
Lactose agar plate, anaerobic, 37 C.	53,000 B. coli	580,000 B. coli	160,000 B. coli	520,000 B. coli	[illegible] B. coli
Spores, aerobic, 37 C.	0	0	280	20	[illegible]
Spores, anaerobic, 37 C.	85	2	20	0	[illegible]
Lactose bile ferm. tube: Gas	65	60	28	70	
Lactose bile ferm. tube: Sediment	Peptolytic	Putrefactive	Putrefactive	Peptolytic	
Gelatin stab, 21 C.	++++	±	±	±	±
Loeffler serum medium, 37 C.	++	±	+	++	−
Sugar broth ferm.: Dextrose	40	40	20	20	8
Sugar broth ferm.: Lactose	35	20	25	60	6
Sugar broth ferm.: Saccharose	25	15	25	28	10
Acetic acid glucose broth N/20	−	±	+	++	++
Acetic acid glucose broth N/10	−	−	−	−	−
Acetic acid glucose broth N/5	−	−	−	−	−
Total gram-positive bacteria	48.0	42.6	58.0	17.3	[illegible]
Total gram-negative bacilli	52.0	57.4	50.0	82.6	[illegible]
Total aciduric bacilli	10.6	30.6	41.9	17.3	[illegible]
Total streptococci	2.1	2.1	1.3	[illegible]	[illegible]
Total spores	0.0	0.0	0.0	0.0	[illegible]
Diet in grams: Protein	Milk	Milk (largely)	92.6†	[illegible]†	[illegible]
Diet in grams: Fat			210.2	[illegible]	[illegible]
Diet in grams: Carbohydrate			161.6	[illegible]	[illegible]
Temperature	104.5	104.5	[illegible]	[illegible]	[illegible]

† Average past 5 days.

* See page 34.

Torello D., Adult, Admitted Nov. 4, 1913, to New York Hospital, in Fourth Week of Disease. Convalescent, Nov. 12, 1913. Mild Typhoid.

Date	Nov. 4	Nov. 8	Nov. 12	Nov. 4	Nov. 8	Nov. 12	Nov. 15
Character of stool	Formed (moist)	Pultaceous	Formed (moist)	Formed (rather dry)	Formed (very dry)	Formed (rather dry)	Semi-formed (moist)
Endo plate	+++ Streptococcus B. coli	++++ Streptococcus B. coli B. pyocyaneus	++++ B. coli Streptococcus B. typhosus	+++ B. coli	+++++ Streptococcus B. paracoli B. coli	++++ Streptococcus B. paracoli B. coli	+++ B. coli Streptococcus B. paracoli
Typhoid bacillus	—	—	+	—	—	—	—
Sugar-free agar plate, aerobic, 37 C.	78,000	130,000	1,300,000	535,000	125,000	35,000	1,500,000
Lactose agar plate, anaerobic, 37 C.	910,000 B. acidophilus	475,000 B. acidophilus	1,300 000 B. coli	535,000 B. coli	90,000 B. coli	57,000 B. coli	1 200,000 Streptococcus
Gelatin plate, aerobic, 21 C.	100,000	195,000	1,250,000	795,000	150,000	11,000	1,400,000
Spores, aerobic, 37 C.	0	1	2	0	0	1	
Spores, anaerobic, 37 C.	7	0	0	0	0	0	
Lactose bile fermentation tube: Gas	50	20	35	85	26	62	70
Lactose bile fermentation tube: Sediment	Fermentative	Fermentative	Fermentative	Putrefactive	Peptolytic	Peptolytic	Peptolytic
Gelatin stab 21 C.	+	+	±	±	—	±	+
Loeffler serum slant, 37 C.	++	—	±	—	—	±	±
Sugar broth fermentation: Dextrose	30	45	62	50	20	40	50
Sugar broth fermentation: Lactose	18	20	45	38	17	30	35
Sugar broth fermentation: Saccharose	5	12	42	18	8	27	15
Milk fermentation: Gas	10	5	10	40	50	10	20
Milk fermentation: Peptonization	--	—	—	+	+	—	—
Acetic acid glucose broth N/20	++++	—	++	+	++	—	—
Acetic acid glucose broth N/10	++++	—	—	—	1	—	—
Acetic acid glucose broth N/5	1	—	—	—	—	—	—
Smear from stool: Total gram-positive bacteria	99.3	88.6	81.0	41.3	47.0	42.6	48.3
Smear from stool: Total gram-negative bacilli	0.7	11.4	19.0	58.7	53.0	57.4	51.7
Smear from stool: Total aciduric bacilli	92.8	79.8	76.2	33.6	61.2	37.2	44.6
Smear from stool: Total streptococci	6.3	3.3	1.3	3.0	1.3	5.6	3.6
Smear from stool: Total spores	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Diet in grams: Protein, Fat, Carbohydrate	History of milk diet with broth for 2 weeks	Daily average of 48 oz. of milk	Daily average of 44.5 oz. of milk	Milk diet since Nov. 1	Daily average of 54 oz. of milk	Daily average of 53 oz. of milk	Daily average of 56 oz. of milk
Temperature	102.8	100	98.4	103	103	99	99

GENERAL OBSERVATIONS

Aside from general observations in regard to the various types of intestinal flora which one may find associated with infection by the typhoid bacillus, several problems of a more special nature have been considered during the course of this investigation: How readily and to what degree can a typhoidal fecal flora be changed in character in response to variations in the constitution of the diet? Do patients with a particular type of fecal flora tolerate with special facility the high calory diet containing large amounts of carbohydrate? What is the effect of liberal amounts of carbohydrate on the intestinal bacteria? What effect has a diet containing much fat on the fecal flora? May an initial unfavorable fecal flora be changed to a favorable type through the use of an appropriate diet? Do patients with an initial favorable type of fecal flora exhibit, on the average, an enhanced resistance to the typical course of typhoid fever?

In respect to some of these points, the evidence has been definite, whereas for others the amount of material has been sufficient to offer no more than suggestions which will be followed out as far as possible in subsequent work.

Before entering upon a discussion of these questions, it is well to review briefly the nature of the intestinal bacterial conditions, as revealed by an examination of the stools, which conditions may be encountered in man after the period of early adolescence. One may find a flora of marked fermentative propensities. In such cases there is a persistence, in a somewhat modified form, of the flora frequently encountered in normal childhood. Bacteria of the bacillus acidophilus type are present in large numbers and may even be the dominant organisms encountered in an examination of the stools. These bacilli utilize little protein in their metabolism, but split many carbohydrates actively with the formation of much lactic acid and without gas production. Associated with these aciduric bacteria are bacilli of the colon group and moderate numbers of streptococci. There are few or no, spores to be seen in gram-stained smears, in which the majority of the organisms are positive to the stain, and few, or no, putrefactive bacteria are revealed by cultural tests. Strong growths occur in the acetic acid glucose broth tubes, while the liquefying action on gelatin is generally moderate, and on Loeffler serum medium is slight. The best single indicator of such a flora is the appearance of the anaerobic lactose agar plates of the peculiar clusters of colonies with a clouding of the medium, which has been described in detail in the preceding section. The persistence of such a purely fermentative flora is probably comparatively rare in adults on the ordinary mixed diet, but, on the other hand, adult individuals, in whom such a flora may be established through a diet for a brief period of large amounts of carbohydrate with reduced proportions of fat and protein, are not infrequently encountered. Examples of such a fermentative flora are detailed in Table 2.

At the other extreme, we find individuals who harbor an obligate putrefactive intestinal flora. In such a flora, the aciduric types are absent, the colon

bacilli are reduced in numbers, and the dominant organisms are a variety of anaerobic bacteria, many of which are spore bearing and all of which are either proteolytic or peptolytic. Streptococci and single cocci may be present in large numbers. As contrasted with a fermentative type, a putrefactive flora is notable for the markedly greater variety of bacterial species. In a cultural examination of such stools, no growth occurs in the acid broth tubes, gelatin and solidified serum are generally rapidly liquefied, and many colonies appear on anaerobic plates seeded from a suitable dilution of the heated emulsion of the feces. Such a flora, as Distaso[16] has pointed out, is commonly found among persons subject to chronic constipation associated with intestinal stasis.

Between these two extremes are all gradations of which we may designate the central type as "facultative." In the typical facultative flora, the dominant organisms are bacilli of the colon type and the majority of bacteria in a direct smear from the feces are negative to the gram stain. The aciduric bacteria are present, but in small numbers, and the same is true of spore-bearing bacilli. Liquefying types and coccal forms may be fairly well represented. Such a flora is called "facultative" because the dominant bacteria may act either in a fermentative or a putrefactive manner in response to the conditions in the intestinal tract and the nature of the pabulum offered them. As has been demonstrated by Kendall and his co-workers,[17] bacilli of the colon and proteus groups, in the presence of a suitable carbohydrate, attack the sugar in preference to the protein that may be present in the medium, but, in the absence of carbohydrate, members of these and other groups become peptolytic and may give rise to indol and other supposedly toxic bodies. The intestinal bacterial conditions in the majority of the typhoid cases investigated soon after entrance into the hospital were of this facultative type, in some instances verging strongly toward the putrefactive type (Table 4). In fact, the great majority of adults in ordinarily good health will be found to harbor such floras.

These rather well-known facts in regard to the various types of intestinal flora have been reviewed because they have an important bearing on the nature of the changes in the numbers and types of the bacteria which followed the use of various diets.

All of these typhoid patients, when placed on the high calory diet, were given at first comparatively large amounts of carbohydrate often in considerable measure in the form of lactose, together with much smaller amounts of fat and protein. As has been observed, it might be anticipated that this preponderance of carbohydrate in the diet would exert a sparing action on the bacterial protein metabolism and would tend to convert the intestinal flora to a purely fermentative type. The change was well marked in certain cases and less so in others; the degree of substitution depended largely on the type of flora which was present at the initiation of the attack. From time to time, the proportions of the fat and carbohydrate, and to a less degree of the protein, were varied and the effect on the flora noted.

16. Centralbl. f. Bakteriol., Abt. 1, O., 1912, 62, p. 432.
17. Jour. Am. Chem. Soc., 1913, 35, p. 1201.

The studies of Herter and Kendall,[5] on the changes in the intestinal flora of monkeys in response to various diets, were the first attempts in the determination the nature and degree of the substitution of bacterial types induced by abrupt variations in the chemical composition of the food. In these experiments, the animals were fed for one or two weeks on a diet consisting almost entirely of protein and then shifted abruptly to a diet consisting very largely of carbohydrate. On the protein diet the flora consisted largely of gram-positive proteolytic or peptolytic bacteria, many of them spore bearing, also many of the colon and coccal types. After a few days of feeding with the carbohydrate diet, this markedly putrefactive flora underwent a rapid change to one of a fermentative type, resembling strikingly that of a human nursling in the predominance of the bacillus acidophilus and the bacillus bifidus types. The change of flora apparently was mainly due to the influence of the carbohydrate. Recently Rettger and Horton[18] have published a somewhat similar study with white rats as the subjects. The intestinal floras of rats on an ordinary mixed diet were compared with the flora of rats on a special diet, which was being used in nutrition experiments by Osborne and Mendel and which consisted of a pure protein (18 percent), protein-free milk (28 percent), starch (29 percent), and lard (25 percent). On the special diet, the flora became simplified and consisted largely of gram-positive bacteria of the acidophilus and bifidus groups with a reduction in the number of colon bacilli.

Bacteriological studies of this character have been extended to man only in a very few instances. Herter[5] observed that, after the addition of 100-200 gm. of cane sugar daily to the ordinary mixed diet of an adult normal human subject, the feces became soft, acid in reaction and odor, and the numbers of bacteria of the acidophilus type increased. MacNeal and his associates conducted an elaborate study of the bacterial elements in the stools of twelve normal adults placed on a definite, weighed diet, but apparently no attempt was made to correlate changes in the flora with chemical variations in the food administered. Kendall has recently attempted to change the "obligate dysentery flora" of infants, consisting of the bacillus dysenteriae, the bacillus coli, and streptococcus, which is potentially putrefactive, to one of a purely fermentative type through the feeding of lactose. In his favorable cases, he noted a gradual disappearance of these gram-negative forms and of streptococci associated with the gradual appearance of the bacillus bifidus and the bacillus acidophilus.

DISCUSSION OF CASES

In Table 1 are detailed the changes in bacterial types which may occur as the result of liberal carbohydrate feeding in typhoidal patients with an initial favorable "facultative" intestinal flora. In these and certain other patients, the first bacterial response to a diet of 2,000-3,000 calories with the carbohydrate portion amounting to 250-300 gm. was a great increase in the numbers of colon bacilli or streptococci, or, in some instances, of both types. Within two weeks, however, the bacillus acidophilus had increased enormously until, in one case, it apparently outnumbered other viable bacteria about 500 to 1. This marked increase of the bacillus acidophilus is shown, not

18. Centralbl. f. Bakteriol., Abt. I, etc., 1915, [illegible]

only by a comparison of the counts from the anaerobic lactose agar plates with those from the aerobic gelatin or sugar-free agar plates, but also by the vigorous growths obtained in the acetic acid glucose broth tubes and by the direct counts from the gram-stained fecal smears. With both the patients (John T. and Enrico P.) these smears had become markedly gram-positive within two weeks and 97 percent of the bacteria seen were of the acidophilus type (John T. and Enrico P., Table 1). In the case of Enrico P., an examination of the sediment from the lactose broth fermentation tube showed that the bacillus bifidus was well represented in the stools, and it is possible that some of the bacteria classified as B. acidophilus in the count from the fecal smear were actually B. bifidus without the swollen or bifid end. The results with these patients prove that, even in adults and in conjunction with an inflammatory condition of the intestinal tract, the flora may be changed through a liberal carbohydrate diet to such a degree that, in many respects, it closely resembles the fermentative and comparatively non-gas-producing flora of the nursling.

In this series of cases, there was none exhibiting in the first examinations a putrefactive flora of the extreme type. There were, however, several examples of a fairly marked putrefactive type, observations on three of which are detailed in Table 4. Such of those cases as were examined soon after admission to the hospital did not reveal the presence of the bacillus acidophilus in the stools; on the other hand, bacteria liquefying gelatin and Loeffler's serum medium were apparently abundant, the sediments in the lactose bile fermentation tubes were putrefactive in type and a comparatively large number of spores developed on the anaerobic lactose agar plates. One of these patients (James C.) died soon after admission to the hospital and another (Thomas F.) could not be placed on more than a moderately high calory diet during the first two weeks in the hospital. As the amount of carbohydrate was gradually increased, the intestinal flora of this patient changed from a putrefactive to a moderately fermentative type. In the case of Emil C. (Table 4) altho the issue is somewhat confused by the alternation at one stage of a high fat with a high carbohydrate diet, practically the same degree of change is revealed. In Table 3 are detailed the results with two patients (Howard F. and Charles F.) of definite, but somewhat less, putrefactive tendencies than the ones just mentioned. Neither of them could tolerate liberal feeding for nearly a month after admission to

the hospital. On the comparatively moderate amounts of carbohydrate which were given them during the first three weeks, there was, at first, some increase in the numbers of colon bacilli and streptococci followed by a moderate multiplication of the bacillus acidophilus, as indicated by the increased growth in the acetic acid glucose broth tubes and the differential counts from the fecal smears. Toward the end of the period of the observation, the amount of carbohydrate fed was greatly increased, but even under these conditions there was nothing like the substitution of floras which was revealed so strikingly for the two cases reported in Table 1.

The increase in the acidophilus types, which are believed to multiply principally in the large intestine and which are dependent upon carbohydrate in their metabolism, would seem to indicate that the amount of carbohydrate given to most of these cases was sufficient to encourage the bacilli of the colon group to exercise their fermentative, rather than their putrefactive, propensities. Du Bois[19] determined, in a study of the food absorption in typhoid fever, that when carbohydrate was given in amounts under 300 gm. a day, it was present in the stools only in traces, if at all, whereas, when amounts over 300 gm. were fed, the stools sometimes contained 2-3 gm. of reducing bodies. These results, it should be observed, were obtained with material which had passed through the whole length of the intestinal tract and had been subjected not only to absorption but to the action of fermentative bacteria. It seems altogether probable that with the large amounts of lactose fed in addition to other carbohydrates some of this sugar may have reached the level of the colon in sufficient quantity to exert a sparing action in that locality on the bacterial nitrogen metabolism. I have found that it is necessary to add only very small amounts of dextrose to Dunham's peptone medium in order to prevent the formation of indol by colon bacilli. Seeding tubes with the bacillus coli communis and also with the bacillus coli communior, indol had formed in medium containing 0.1 percent of dextrose, but not in that containing 0.2 percent. This same result was obtained after 3 days' incubation at 37 C. and also after seven days' incubation. It is evident, then, that the presence of only slight amounts of a usable carbohydrate are necessary in order to prevent the peptolytic action of the bacillus coli.

19. Arch. Int. Med., 1912, 10, p. 177.

In six patients, the initial flora, that is, the one observed before carbohydrate had been given in liberal amount or at most for not more than a few days, was markedly fermentative in type. The bacterial findings in four of these cases are given in detail in Table 2. The dominant bacteria in the stools were from the start of the bacillus acidophilus type and there was a notable absence of putrefactive organisms. Accordingly, there was present in these cases at the beginning of the attack a type of flora which was attained in certain patients, with an initial facultative type, only after two weeks or more of feeding with large amounts of carbohydrate. As might be anticipated in these cases, the high carbohydrate diet maintained and accentuated this purely fermentative and relatively non-gas-producing flora. It seems not unlikely that if perforation should occur in cases with a flora of this character, the results might be less disastrous than for patients harboring an intestinal flora of putrefactive propensities.

As far as the incidence of the typhoidal infection is concerned, there was no evidence that it was favored by one type of intestinal flora more than by another; this series of cases exhibited intestinal bacterial conditions in the early stages of the attack which ranged from the putrefactive to a benign type of fermentative flora. Of course, the conditions at the time of primary infection may only be surmised, altho there is no reason to suppose that they differed markedly from what was encountered on their admission to the hospital.

With most of the cases especially in the earlier stages of the feeding, the daily amount of fat in the diet ranged from 75-125 gm. The effect on the cultural results of a sudden shift to a diet containing a larger ration of fat apparently depended upon whether the carbohydrate allowance was simultaneously decreased or was maintained at a high level. If, after a fermentative flora had been established, the fat in the diet was increased to about 200 gm. and the carbohydrate reduced to the same amount or less, there occurred a reduction in the number of viable bacteria in the stool which generally included the colon types, but was especially striking as regards the aciduric types. In the case of Christian M. (Table 2) the bacillus acidophilus was apparently almost eliminated, but on a return to the high carbohydrate feeding, it had again regained its dominance within three days. It should be observed that the amount of protein was kept at the same level in the diet of this case and only the fat and carbohydrate were varied in amount. If the amount of fat was increased to about

200 gm. daily, but, at the same time, the carbohydrate was maintained at 300-400 gm., any reduction in the number of viable bacteria in the stools which might occur concerned alone the bacilli of the colon group and the numbers of acidophilus bacilli remained at a high level (Table 2, Anthony G., Table 1, John T.). During the convalescence of Enrico P., about equal amounts by weight of protein, fat, and carbohydrate were given for a period of ten days. This resulted in an almost complete elimination of the bacillus acidophilus and an increase in the numbers of colon bacilli, the appearance of the direct smear from the feces changing from a predominately gram-positive type to an equally markedly gram-negative one (Table 1). I intend to enquire farther into the effect of varying the amounts of fat and carbohydrate on the intestinal flora. My results thus far indicate that fat is not potentially related to carbohydrate from a bacterial standpoint as has been suggested by Kendall.[3]

The stools of patients on a high carbohydrate diet were almost always moist and semi-formed, whereas those from patients on a high fat diet were formed and generally rather dry. The question arises, then, whether the cultural results with the stools of patients on a high fat diet reflect the bacterial conditions in the lower ileum and colon or whether the inspissation of such stools in the passage through the lower colon and rectum was the factor responsible for the variations in the numbers of viable bacteria. As regards the colon bacillus, altho the increase and decrease of their colonies could not always be correlated with the degree of moisture in the movement, yet frequently a low count was associated with a rather dry formed stool as MacNeal and his associates found in their series of examinations. On the other hand, the inspissation of the stool seemed to bear no relation to the numbers of the bacillus acidophilus which might appear in the cultures, but their increase and decrease, as revealed in the stool examinations, were governed entirely by the relative and actual amount of carbohydrate in the diet. It is in fact well established that these aciduric bacilli withstand drying successfully. Accordingly, there are good grounds for the assumption that the numbers of the bacillus acidophilus revealed in the cultures constitute a true index of their numbers at the level of the intestinal tract which they frequent.

It seemed advisable to determine whether or not typhoid patients, given a purely milk diet, would exhibit changes in their fecal flora similar to those which were observed with patients on a high calory

diet with a liberal proportion of carbohydrate. Through the courtesy of Dr. Lewis A. Conner, I had the opportunity of examining bacteriologically the stools of three such patients at the New York Hospital. Two of these patients (Table 5, Joseph P. and Torello D.) were given daily an average of about 52 oz. of milk. This constitutes a liberal feeding of milk, but, nevertheless, is a low calory diet. This amount was probably equivalent to about 975 calories, of which the carbohydrate amounted to approximately 70 gm., the fat to 52 gm., and the protein to 45.5 gm. This amount constituted no more than one-third to one-half the amount usually given in the Coleman high calory diet, even in the early stages of the disease. It would seem unlikely that this allowance of carbohydrate would prove sufficient to encourage or maintain a benign fermentative flora, and such proved to be the fact. In one of these milk-fed cases (Table 5, Joseph P.) a distinctively fermentative flora was encountered at the beginning of the trial, but after four days of milk feeding the organisms of the bacillus acidophilus type had decreased greatly in numbers and in eight days they were almost entirely replaced by colon and streptococcus types. The fecal flora had thus undergone a transformation to one potentially peptolytic and indol-forming. As there was a history of a milk diet for this patient for two weeks before admission to the hospital, about 3 weeks were apparently required to bring about the marked change in the character of the flora. In the second of these cases (Torello D.), during fifteen days on a milk diet, the initial moderately putrefactive type of flora was transformed to a peptolytic, but not to a purely fermentative, type. Toward the end of the period, there was a marked increase in the number of streptococci. A much greater variety of bacteria was observed in the gram-stained smears and also on the culture plates seeded from the stools of these two milk-fed patients than was the case with patients fed liberal amounts of carbohydrate. In fact, the simplification of the flora, following a course of high carbohydrate feeding, was a very striking feature. On the milk diet, colon types which acted slightly or not at all on lactose were common, a state of affairs which suggests an explanation of the fact that greater volumes of gas were formed in the dextrose than in the lactose fermentation tubes. The stool examination of the third patient on the milk diet revealed the same general features as the other two. At the time of taking the specimen, she had been four days on the milk diet and there was, in addition, a history of milk feeding for one week before admission.

As far as typhoidal cases are concerned, the bacteriological examination of these stools would seem to indicate that a daily diet containing such a small amount of carbohydrate as 70 gm. with about two-thirds as much each of fat and protein is not a combination which will encourage the development in the adult of a non-indol forming intestinal flora. On the other hand, the best results were attained, as regards the development and maintenance of a fermentative flora, when carbohydrate to the amount of 300-400 gm. was given daily. It is manifestly impossible to feed this amount of carbohydrate when milk alone constitutes the diet.

Altho the number of cases examined was too few to offer more than a suggestion, it is of interest to note that with five of six patients exhibiting a markedly fermentative flora, with the bacillus acidophilus dominant either before a diet containing much carbohydrate had been given at all or for not more than a few days, the infection ran a comparatively mild and short course. With the sixth patient exhibiting such a flora (Morris S., Table 2) altho the febrile period extended through two relapses, the patient's state of nourishment remained very good and convalescence was rapid. On the other hand, patients who suffered the severer attacks of the fever were those who harbored an initial flora of putrefactive tendency. Herter observed that individuals in middle life with an intestinal flora of the adolescent fermentative type were usually well nourished, free from nervous disturbances, and possessed of much reserve energy. When such individuals contract typhoid fever it seems not unlikely that their probable lifelong freedom from the continued absorption of the toxic products of a putrefactive fecal flora enables them to withstand the systemic invasion of the typhoid bacillus with greater success than can individuals who have been subjected to auto-intoxication for many years. I do not wish to lay stress on this suggestion at this time but it is well known that attacks of typhoid fever among children, in whom as a class auto-intoxication of intestinal origin is not a frequent factor, are often mild.

BACTERIAL OBSERVATIONS

Typhoid Bacillus.—A survey of the tables shows that the typhoid bacillus was isolated less frequently from this series of cases on the high calory diet than has been reported from certain other groups of cases on a less liberal diet. From a few patients, this bacillus was not isolated at all in examinations of the stools conducted at intervals dur-

ing almost the whole course of the fever and through convalescence. In almost every examination, from three to five Endo plates of the large size were made from graded dilutions of the stool and every suspicious colony was subjected to differential tests. In this procedure, a slant of Russell's double sugar medium was seeded from the colony in question and, if the growth and color reaction proved at all characteristic of the bacillus typhosus, the identification was established through agglutination tests. In certain instances where the typhoid bacillus was not recovered from stools with the Endo plate method, it was isolated by Dr. Oscar Teague, using a selective plating method. These comparative tests were carried out on only a comparatively few stools, and where success was attained the fact is indicated in the tables by an asterisk. Pratt, Peabody, and Long[20] observed no difference in the frequency of the isolation of the typhoid bacillus from the stools of patients on a milk diet and those on a more liberal diet, the amount and constitution of which is not definitely stated. A possible explanation of the infrequency of the isolation of typhoid bacilli from the stools of these patients on the high calory diet may be found in an unusual degree of acidity in the lower ileum and the colon, arising in that locality through the bacterial splitting of the large amounts of carbohydrate. The typhoid bacilli, eliminated from the gall-bladder, in their passage through the intestinal tract would probably find this environment unfavorable and the survivors might be so few as to be crowded out on the Endo plates by colonies of the ordinary intestinal bacteria. In this connection, it may be mentioned that Kendall noted a tendency of dysentery bacilli to disappear from the stools of infants suffering from summer diarrhea when lactose was fed in quantity sufficient to change the flora from a putrefactive to a fermentative type.

Streptococci.—Without regard to the general character of the flora and the nature of the diet, streptococci were present in considerable numbers in most of the stools. Streptococcus is apparently an organism which can adapt itself readily to changing and diverse conditions. On a liberal carbohydrate diet, streptococci generally increased rapidly during the first week or so but were largely replaced later by the bacillus acidophilus (Table 1). With a milk diet, the streptococci increased rapidly and continued to be present in large numbers in the stools. On the Endo plates, the streptococcus colonies ranged

20. Jour. Amer. Med. Assn., 1907, 49, p. 846.

from white to a deep scarlet. The white or pink colonies generally outnumbered the red and, by cultural tests, were found either to be raffinose fermenters and, hence, possibly to be grouped with the streptococcus salivarius, or to conform to the general type of the streptococcus lacticus. Certain of the deep red streptococcus colonies, which were subjected to differential tests, were found to be mannite splitters and in other respects to resemble the type, Str. fecalis.

Bacillus Acidophilus.—Rahe[21] has investigated the biological characteristics of the bacillus acidophilus strains recovered from the stools of these typhoid cases and also cultures isolated from other sources. He has determined that the bacillus acidophilus group may be separated from the bacillus bulgaricus group, in a measure, through the type of colony formation on glucose oleate agar, but especially by the fact that the bacillus acidophilus ferments maltose whereas the bacillus bulgaricus is not able to split this sugar. The bacilli of the bacillus acidophilus type, according to his findings, may be further divided into two groups: those which clot milk within six days at 37 C., and those which do not clot milk within that period, and probably not at all. In a study of the strains of the bacillus acidophilus isolated from these typhoidal stools, it was found that in instances where the first examination from a patient disclosed large numbers of the bacillus acidophilus in the stool or where this bacillus became the dominant organism as the result of feeding large amounts of carbohydrate, the bacillus acidophilus present belonged in his Group 2, which clots milk readily, whereas cultures isolated from cases in which bacillus did not increase markedly as the result of such feeding belonged in his Group 3, which does not clot milk within six days' incubation.

Other Bacteria.—The bacillus pyocyaneus was found in the stools during the earlier stages of the disease in a few of the cases. These cases included both mild and severe types. This bacillus, in each instance, disappeared from the feces after the patient had been fed for a time with a high carbohydrate diet. No proteus like colonies were observed on any of the plates. As has been mentioned, spores occurred in relatively small numbers in the most of the stools. In a number of the patients to whom carbohydrate was given in large amount, practically no spores developed on either the aerobic or anaerobic plates, but following a high-fat diet colonies developing from spores appeared in increased numbers on the aerobic plates. These were mostly thin,

21. Jour. Infect. Dis., 1914, 15, p. 141.

dry, spreading, and frosted in appearance. Such spores as developed on the anaerobic plates were largely of the bacillus aerogenes capsulatus type. Spores of the bacillus putrificus type were rarely observed in the smears from the feces. Louis-Melikov[22] has emphasized the supposed part played by anaerobic proteolytic bacilli, resembling the bacillus perfringens, in the production of the intestinal lesions. He has designated them "satellite bacilli." In our series of cases, neither the cultures nor the examinations of the smears has revealed unusual numbers of the spore-bearing bacilli of the type he describes. In the smears from the stools of some of the patients, many yeasts were observed. In one instance (Table 2, Morris S.), the frothy character of the stools was apparently due to them.

SUMMARY

In a series of typhoid cases, the intestinal flora as revealed by examinations of the stools soon after the admission of the patients to the hospital was not uniform or specific in type, but exhibited variations that might be observed in a series of supposedly normal individuals. Apparently, the character of the intestinal flora is not a factor concerned in the determination of susceptibility to typhoidal infection.

On a diet consisting of a daily average of 50-100 gm. of protein, 75-100 gm. of fat, and 250-300 gm. of carbohydrate, including lactose, the intestinal flora tended to become simplified in regard to the variety of bacterial types observed in direct gram-stained smears from the feces and in cultures and to become converted into a fermentative type in which the dominant organism was Bacillus acidophilus. The degree of this transformation was dependent largely upon the type of flora which was present at the onset of the disease. When the flora showed a definite putrefactive tendency, the change did not extend farther than the elimination of the obligate putrefactive organisms and a moderate development of the aciduric types; while, with a more favorable initial flora, the change was of so radical a type that the stools finally resembled those of the first few years of normal infancy in the dominance of the bacillus acidophilus and even in the presence of the bacillus bifidus. Such a flora is not an obligate fermentative one and under the conditions of feeding is not capable of forming indol. Observations on a few patients given a purely milk diet indi-

22. Ann. de l'Inst. Pasteur, 1913, 27, p. 541.

cated that this food alone does not bring about this transformation of the intestinal flora to such a degree as that which may follow a more liberal carbohydrate feeding.

Patients exhibiting an initial fermentative flora of the aciduric type adapted themselves more readily to the high calory diet of Coleman, as shown especially by a comparative freedom of distention, than did patients with floras of putrefactive tendencies; in such patients, the diseases showed a marked tendency to run a mild course.

The typhoid bacillus was isolated from the stools of these patients on a high calory diet less frequently than has been reported for other series of cases in which the feeding was less liberal.

THE NERVE CONTROL OF THE THYROID GLAND

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THE NERVE CONTROL OF THE THYROID GLAND[1]

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TWO mechanisms are established within the animal body by means of which the functions of its different parts are co-ordinated. One of these acts through the medium of the nervous system, while the other is chemical in nature. The two are, however, interdependent to some degree. The activities of the nervous system in this respect have long been a subject for accurate investigation, but the work of recent years has served to emphasize the importance of the hormones, the chemical messengers by means of which widely separated structures are brought into a harmony of action. At some link in the chain it will be found, however, that the determining influence of the nervous system is predominant.

The thyroid gland is a favorite example of the glands which produce internal secretions. Its functions and pathological conditions are perhaps better known than any others in its class. Variations in its functions are correlated with fairly well-defined clinical conditions. A large number of studies have been made upon the chemical nature of its active substance, and many attempts have been made to show how its activity is regulated, at least in part, by the secretions of other ductless glands.

It seems very probable that the active substance in the thyroid gland contains iodine in combination with protein, and that the physiological activity is in proportion to the quantity of iodine in the combination. We believe that the function of this substance is not performed within the gland itself. The colloid of the gland may be looked upon as reserve material ready for dis-

[1] The expenses of this research were defrayed by the Johnston Livingston Fund for Experimental Therapeutics.

charge into the blood or lymph, through which medium the general tissues of the body are supplied. Some attempts have been made to demonstrate the presence of this active substance in the blood by means of a biological reaction, but none of them have thus far been successful. If one considers how very vascular the gland is, and how small a quantity of the active substance is distributed through the blood leaving the gland in twenty-four hours, it is not surprising that none of these methods have satisfactorily demonstrated the presence of the iodized protein in the circulation.

Recently Asher and Flack[1] have attempted to demonstrate the nerve control of the thyroid. Their experiments are indirect and are so complicated as to leave the matter in great doubt. Their experiments were made upon rabbits, cats and dogs, with a view to determine whether any phenomena could be observed after stimulation of the thyroid nerves which would lead to the conclusion that stimulation had caused an increase in thyroid secretion in the blood. As a means to this end they compared the excitability of the depressor nerve before and after stimulation of the thyroid nerves, and also the effect of a small intravenous injection of adrenalin before and after such stimulation. They conclude that under otherwise exactly similar experimental conditions, a stimulation of the depressor nerve or an intravenous injection of adrenalin was more effectual during a stimulation of the thyroid nerves than shortly before without it. That both these symptoms, viz., increased depressor excitability and more effective action of adrenalin, actually depend upon an inner secretion of the thyroid and not upon any accompanying circumstance of nerve stimulation is proven by the fact that on extirpation of the thyroid the symptoms no longer appeared, and on the other hand intravenous injections of thyroid extracts acted exactly like stimulation of the thyroid nerves. Their experiments are complicated and while suggestive they do not satisfy.

The purpose of the experiments reported in this communication is to give evidence of a different character that the thyroid gland is, at least in part, under nerve control. Briefly, the plan of the experiments was to determine, first, whether the iodine content of the two lobes of the normal thyroid gland from the

same animal is identical; and second, whether stimulation of the nerves leading to one lobe causes a loss in its iodine content. If the two lobes of the normal thyroid are identical in iodine content they may serve as controls of one another. Granting such to be the case it was our hypothesis that stimulation of the nerves to one lobe might cause a change in the iodine content because of the resulting discharge of iodized proteid from the gland.

The method followed in determining the iodine in all the analyses herein reported was the modification of the Baumann process devised in this laboratory by Riggs.[2] Some very slight changes in the technique were made which experience has shown to be valuable in rendering the fusion easier and in lessening the tendency to form iodates to such a degree that many of the fusions were wholly free from such compounds. The procedure in detail was as follows: the fresh glands were carefully trimmed from any adhering tissues and accurately weighed; they were then placed in a nickel crucible and covered with 10 to 15 c.c. of a 50 per cent solution of sodium hydroxide. Heat was carefully applied and the glands disintegrated and finally fused. Such a technique is preferable to the use of the solid hydroxide in that it gives a gradual disintegration of the gland and allows carbonization to take place at a lower temperature. At no time was more than a dull red heat applied and often not that. When the mass had been thoroughly fused, only as much sodium nitrate was slowly added as was necessary to oxydize any small particles of carbonaceous material remaining. After cooling, the fused mass was dissolved with hot water, quantitatively filtered, made up to exactly 100 c.c. and thoroughly mixed. From this point the method of Riggs was followed exactly.

In this laboratory we have found that in determining such small amounts of iodine as is found in dogs' thyroids the method is preferable to that of Hunter,[3] in that the fusion is more easily carried out, and it requires the use of fewer standardized reagents. In Kendall's[4] method the fusion is quite similar except that he uses larger amounts of sodium hydroxide and potassium nitrate, and reduces excess by the use of gallic acid which in itself causes trouble if too much is added. His method for titration after fusion is more complicated than Riggs' colorimetric method and,

in this laboratory, has not been found more accurate when using minute quantities of iodine.

For the purpose of determining whether the iodine content of the two lobes of the thyroid is the same per gram of fresh gland several animals were killed and the glands immediately dissected out and subjected to the method outlined above. No particular selection was followed with reference to the animals, and in no case was there found any gross pathological condition of the gland. They were the usual animals kept in stock and had been fed the same diet which we have used for a number of years, viz. boiled beef hearts and bread.

Table I shows the results of these analyses:

TABLE I

SHOWING IODINE CONTENT OF THE TWO SEPARATE THYROID LOBES FROM THE SAME ANIMAL

Lobe	Weight of lobe	Milligrams Iodine found	Milligrams Iodine per gram of gland
R	0.763	0.500	0.893
L	0.886	0.550	0.893
R	0.7048	0.475	0.674
L	0.6600	0.450	0.681
R	0.7174	0.700	0.975
L	0.7620	0.750	0.984
R	1.2786	0.900	0.7038
L	1.1400	0.800	0.7041
R	1.0956	1.100	1.004
L	0.9872	1.000	1.017
R	0.9810	1.440	1.468
L	0.8154	1.200	1.472
R	0.3602	0.132	0.3664
L	0.4020	0.145	0.3607

From the table given it is evident that the iodine content varies widely in the different animals, but the two lobes are wonderfully constant, the difference shown falling within the limit of error of the analytical method. The largest difference

noted is found in the third pair of glands, in which there is a difference of only .009 mgms. per gram of fresh gland.

The figures are based upon the weight of the fresh gland rather than of the dried substance, because most analyses of the iodine content of these glands recorded in the literature are so expressed, and furthermore because it is probably more accurate. It is not a simple matter to dry the glands to constant weight and transfer the dried powder without loss to a fusion crucible, and the fusion of the moist gland in the manner described above is much simpler.

It seems evident from the analysis quoted that one lobe of the thyroid may serve as control of the other provided both lobes are normal.

The method of conducting the stimulation experiments was as follows: The stimulation in each instance was electrical and was obtained by connecting three dry cells in series for the primary current. This current was connected with the ratchet wheel of a clock in such a manner as to permit a momentary stimulus every ten seconds. The current from the secondary coil was so regulated that only a faint stimulus was given to the moist tongue of the operator.

The nerve supply to the thyroid probably has its origin in filaments from the sympathetic. In man a nerve filament can be demonstrated to arise from the superior cervical ganglion of the sympathetic, and to follow more or less closely the course of the superior thyroid artery, and to terminate in the upper anterior portion of the superior pole of the thyroid. In addition there are microscopic filaments from the sympathetic which can be demonstrated to enter the gland in the walls of both the superior and inferior thyroid arteries and sympathetic filaments are apparent around each of the thyroid vescicles.[5]

In the dog the same nerve filament exists entering each of the separate thyroid lobes near the outer superior pole, but the cervical sympathetic and the pneumogastric nerves form one strand and the apparent nerve supply of the gland cannot be proved by dissection, as in man and the cat, to arise only from the sympathetic. Nevertheless it is possible that the dog's anatomy is not different from that of the cat and man in this respect.

In these experiments ether anaesthesia was used in each case and was administered through a bottle with a cannula in the trachea. The method of arranging the stimulation was varied in the different experiments.

Stimulation Experiments — Series I.

In these experiments one lobe of the gland was removed immediately before the stimulation was begun. This lobe served as a control. The vessels of the upper pole of the remaining lobe were carefully separated from the connective tissues and the electrodes were then carefully brought into contact with the vessels and the accompanying nerve filaments. Careful approximation of the electrodes was carried out so that the blood supply and return would not be impaired.

(In the fifth experiment in this series both lobes were left in until the end of the period of stimulation.)

TABLE II

Showing Iodine Per Gram of Control and Stimulated Glands. Series I.

Gland	Wt. of gland grams	Iodine in mgms. per gram	Length of stim.
Control	.485	.2051	2 hrs., 30 m.
Stim.	.417	.1923	
Control	.4062	1.1078	3 hrs.
Stim.	.3717	1.0088	
Control	1.0680	.8895	3 hrs.
Stim.	1.1234	.7566	
Control	1.2280	1.3029	3 hrs.
Stim.	1.2000	1.0417	
Control	.6564	.9012	2 hrs., 30 m.
Stim.	.5770	.7312	

The average loss in iodine was .1351 mgms. per gram of gland, but in that case No. 4, in which the odine content of the control pland was highest, the loss on stimulation was .2612 mgm. iodine ger gram of gland, a loss of approximately 20 per cent. In every

stimulated gland there has been a loss so great as to be beyond the possibility of error in the determination.

Stimulation Experiments — Series II

In these experiments both lobes remained intact throughout the experiment. After the period of stimulation they were both removed and prepared for analysis. The point of stimulation was on the combined vagus and sympathetic. The vagus on the side stimulated was ligated low down on the neck, a careful dissection was made, the superior ganglion exposed, and the nerve cut central to the ganglion. The electrodes were applied to the nerve peripherally to the ganglion. The exposed tissues were covered with cotton moistened with Locke's solution.

TABLE III

Showing Iodine in Milligrams per Gram of Gland after Stimulation Through Nerve

Gland	Wt. of lobe in grams	Iodine in mgms. per gram of gland	Time of Stim.
Control	2.7573	.1813	2 hrs., 30 m.
Stim.	2.5273	.1582	
Control	.6174	.3239	3 hrs.
Stim.	.8126	.1723	
Control	1.0646	1.3620	2 hrs., 15 m.
Stim.	1.2070	1.0270	

The analytical results in this series show a larger percentage loss of iodine than in those quoted in the previous experiment. In the second animal nearly 50 per cent of the iodine in the stimulated lobe was discharged into the blood or lymph during the period of stimulation.

Stimulation Experiments — Series III

In this series the thyroids were both left intact until the termination of the experiment. The combined vagus and sympathetic were isolated about one inch below the thyroid cartilage

and the stimulation which was identical with that used in the previous experiments was applied at this point. The nerve was not cut or ligated in any manner. The circulation through the gland was not impaired or disturbed by the operative procedure.

TABLE IV

Showing Loss of Iodine in Glands Stimulated Through the Intact Nerve

Lobe of gland	Weight in grains	Iodine in mgms. per gram	Loss	Time
Control	.9469	.581		45 min.
Stim.	.8048	.559	.022	
Control	2.8732	2.866		3 hrs.
Stim.	3.1000	1.967	.899	
Control	.5228	2.486		3 hrs.
Stim.	.5008	2.196	.290	
Control	.7450	2.013		3 hrs.
Stim.	.5425	1.843	.170	
Control	.4450	.272		30 min.
Stim.	.6660	.241	.031	
Control	.4738	2.216		3 hrs.
Stim.	.6647	2.031	.185	
Control	.7830	1.277		3½ hrs.
Stim.	.8730	1.027	.250	

Summary

The experimental results may be summarized in the following way:

1. The average difference of iodine, expressed in mgms per gram of fresh gland, between the two thyroid lobes of seven normal dogs was .0055 mgms.

2. The average difference found when the superior vessels, with accompanying nerve fibres, of one lobe were stimulated was .1351 mgms.

3. The average difference found when the stimulus was applied to the vagus peripherally to the superior ganglion, the nerve being cut central to the ganglion and ligated peripherally to the stimulus, was .1699 mgms.

4. The average difference found when stimulus was applied to the intact vagus was .2640 mgms. In series III two glands were stimulated 45 minutes and 30 minutes respectively. If the loss in the remaining five glands which were stimulated three hours is averaged it will be found that a loss of .3588 milligrams per gram of gland occurred.

In no case did we fail to get a loss after stimulation. There seems to be no reasonable doubt from these results that the thyroid is at least in part under nerve control, and that its physiologically active substance is discharged into the circulation in response to a nerve stimulus.

[1] Asher and Flack: Zeitschrift für Biologie, 1911, lv, p. 83.

[2] Riggs: Journal of the American Chemical Society, 1910, xxxii, p. 692.

[3] Hunter: Journal of Biological Chemistry, 1910, vii, p. 321.

[4] Kendall: Journal of the American Chemical Society, 1912, xxxiv, p. 894.

[5] Rhinehart: American Journal of Anatomy, 1912, xiii, p. 91.

AN INVESTIGATION INTO THE FERMENTATIVE ACTIVITIES OF THE ACIDURIC BACTERIA

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The wide use of the bacillus bulgaricus, both topically and by mouth, to control intestinal putrefaction has within the last few years given rise to a number of investigations aiming to give a definite classification to this group of lactic acid bacilli. It would seem that this could be satisfactorily accomplished by a systematic examination of their prominent cultural characteristic, namely, the fermentation of carbohydrates. That this means of separation has not been successfully used heretofore is probably due to the assumption that these bacteria will not grow on the usual laboratory media, though mention is made by the earlier workers of growth in glucose and lactose broth.

According to Grigoroff,[1] the bacillus bulgaricus attacks mannite, saccharose, maltose, and lactose, but not rhamnose, dulcite, or sorbite; according to Cohendy,[2] the bacillus attacks lactose, maltose, saccharose, levulose, and dextrose. In a later work, Bertrand and Duchacek[3] state that this bacillus ferments dextrose, mannite, galactose, levulose, and lactose, but does not attack arabinose, xylose, sorbite, maltose, and saccharose. The lack of agreement exhibited by these findings make them of doubtful value in the identification of this bacillus.

A similar and probably closely related organism is that isolated by Moro from infants' stools and referred to by him as the bacillus acidophilus. The similarity of behavior both biochemically, and as far as was known, culturally, of the acidophilic and bulgaricus groups led several writers, notably Rodella[4] in 1908, and Heinemann and Hefferan[5] in 1909, to conclude that they are identical. Although a survey of the literature and a study of their properties leaves no doubt as to their common membership in the group of non-gas-forming, acid-resisting, lactose-splitting bacteria, there exist, nevertheless, well marked cultural differences that set them apart. These differences form the subject of this paper.

1. *Rev. med. de la Suisse romande*, 1905, 25, p. 714.
2. *Compt. rend. Soc. de biol.*, 1906, 60, p. 558.
3. *Ann. de l'Inst. Pasteur*, 1909, 23, p. 402.
4. *Centralbl. f. Bacteriol.*, Abt. I, O., 1901, 29, p. 717.
5. *Jour. Infect. Dis.*, 1909, 6, p. 304.

The literature relating to the bulgaricus type of bacillus has been so often summarized that a repetition at this time seems unnecessary and the reader interested in that phase of the subject is referred to the articles of Heinemann and Hefferan,[6] White and Avery,[7] and Kuntze.[8] The state of the literature with respect to the bacillus acidophilus is much the same as in the case of the bacillus bulgaricus; it is recognized as a fermentative organism but apparently little is known in regard to its action on different sugars. According to Moro,[9] the bacillus acidophilus is an acid-resisting bacillus that grows poorly in the usual media and forms irregular colonies on agar. It grows at body temperature and also at 20 C. It is an acid former and coagulates milk. The bacillus acidophilus of Finkelstein has essentially the same cultural characteristics as Moro's bacillus with the exception that it does not coagulate milk, as stated by Lehmann and Neumann.[10] Rodella[11] described a strain exhibiting branching and streptobacillus forms which gave colonies of different shapes on agar plates. Mereshkowsky,[12] who studied the distribution of this bacillus in the animal kingdom, recognizes two types of colonies formed by it, a round, compact, even-edged type, which he designates as Type 1, and one with a filamentous border, Type 2. He found no cultural difference between them. He made use of the following reactions in their identification: they live one to three days in 1 to 0.5 per cent acetic or lactic acid broth; they form the two mentioned types of colonies; they are Gram-positive, and they cause agar to become cloudy.

There seems to be no doubt of the wide distribution in nature of these bacteria and there is likewise no question as to their occurrence among the intestinal flora. Podajezky,[13] who isolated them from bottle-fed and breast-fed infants, from a 10-year-old girl and from a man of 34, showed that the bacillus acidophilus exists in the intestinal tract regardless of age and variety of food. Mereshkowsky isolated it from a long series of animals ranging from mollusks to man.

The general characteristics of both the bulgaricus and acidophilus types of bacilli may be summarized as follows: They are bacilli of varying lengths, occur singly or in chains or threads and develop under both aerobic and anaerobic conditions. Typically they stain Gram-positive but old cultures may be Gram-negative. They produce acids from carbohydrates but do not form gas. They do not form spores. They survive and multiply in media containing considerable amounts of acid. Colony formation, the coagulation of milk, and action on carbohydrates are considered in detail further along.

Since Heyman in 1898 used acetic acid in dextrose broth in isolating a bacillus of the acidophilic type, many authors have followed his example and in this investigation a medium of the same general composition has been used, viz.,

6. *Ibid.*
7. *Centralbl. f. Bacteriol.*, I, O., 1910, 25, p. 161.
8. *Ibid.*, II, 1908, p. 161.
9. *Wien. klin. Wchnschr.*, 1900, 5, p. 114.
10. Bacteriologie, München, 1907.
11. *Centralbl. f. Bacteriol.*, I, O., 1901, 29, p. 717.
12. *Ibid.*, 1905, 39, p. 380.
13. Quoted by Mereshkowsky.

meat-peptone broth containing 2 per cent. glucose and 0.3 per cent. acetic acid. A little of the substance under investigation was seeded into a tube of the broth and incubated for forty-eight hours. One-half c.c. of this culture was then seeded into another tube, and again incubated. After a third seeding and incubation, a loopful of the broth was streaked on the surface of an agar plate. The triple seeding was made for the purpose of eliminating bacteria other than those of the type desired. This is the procedure recommended by Kendall.[14] The agar used for plating was of the usual kind containing 1.5 per cent. of agar and 2 per cent. dextrose, but with no adjustment of its acidity. The growth obtained on this agar was fully equal to that on dextrose whey agar. Later the procedure recommended by Salge[15] was adopted and 0.2 per cent. of sodium oleate added to this medium. As this oleate agar gave a better growth than the plain glucose agar described above, it was used throughout the latter part of the work. Its reaction varied from 0.8 to 1 per cent. The agar plates were incubated for forty-eight hours before the colonies were picked off. In a series of about 100 isolations only four gas formers were found; streptococci were few and aside from these and an occasional growth of yeasts, none but acid-resisting bacteria grew on the plates. The sources of material are given in Tables 1 to 3.

Aside from milk, the only medium used in the differential tests was unneutralized meat-peptone sugar-free broth with the addition of various carbohydrates to the amount of 2 per cent. The initial reaction of the broth varied from +2.2 to +2.8 per cent. Since this variation could not have affected the activity of the bacteria to an appreciable extent, and since the composition of the original broth could not be controlled, nor could a slight degree of hydrolysis be prevented during sterilization and incubation, the various lots of broth were considered in close enough accord for the purpose of this investigation. The broth was sterilized in bulk in the Arnold sterilizer and care was taken to prevent more than a very slight hydrolysis of the carbohydrate. The medium was then tubed in 9 c.c. amounts, and after a preliminary incubation test for sterility the tubes were seeded with a loopful of a forty-eight hour culture of the organisms to be examined, and incubated for five days at 37 C. Five c.c. of each culture were then titrated in the cold against N/10 NaOH, using phenolphthalein as an indicator. It will be observed that this medium is in every sense a "usual" one and it is difficult to see why it has not been used before for the purpose of differentiation. It is true that in the case of organisms of the bulgaricus type visible growth may not appear before forty-eight hours, but it is likewise true that in the presence of suitable sugars there is a measurable degree of fermentation at or before this time. Cultures in this broth have remained viable for a month or longer when kept in the ice chest.

Milk was tubed in 10 c.c. amounts and incubated for a period of 10 days. When clotting occurred the contents of the tube were worked up into a smooth mass and care taken to avoid air bubbles in pipetting the medium for titration. In Tables 1, 2 and 3 Mereshkowsky's terms have been used to indicate the type of colony formed, and the bacilli themselves are classified according to their reaction to Löffler's stain as suggested by White and Avery. In these tables the acid is calculated on the basis of 94 per cent. lactic and 6 per cent. volatile acid. These are the values determined by Heinemann and Hefferan.[16]

14. *Jour. Med. Research*, 1910, 22, p. 153. In this article Kendall suggests the term "aciduric" to take the place of "acidophilic."
15. *Jahrb. f. Kinderh.*, 1904, 59, p. 309.
16. *Jour. Infect. Dis.*, 1909, 6, p. 304.

Table 1 shows that the bacteria of this group all form colonies that correspond to Mereshkowsky's Type 2. Milk was coagulated as a rule before twenty-four hours at 37 C., and there was a large production of lactic acid during the six-day period. The action on maltose was so slight that it may be regarded as negative, and in one case there is a distinct reduction in acidity. With saccharose there was a slight acid production in two instances and a marked one in a third. In the column headed "Type of B. Caucasicum (White and Avery)" the strains are classified according to their reaction to Löffler's methylen blue. According to these authors those that are rapid fer-

TABLE 1.

FERMENTATIVE ACTIVITY OF BACTERIA OF GROUP 1

Culture	Source	Type of Colony (Mereshkowsky)	Type of B. Caucasicum (White and Avery)	Total Acidity Percentage of Normal Acid Six Days in Milk	Percentage of Fixed Acid in Terms of Lactic Acid	Percentage of Normal Acid in Maltose Broth, Five Days	Percentage of Normal Acid in Saccharose Broth, Five Days
Museum	Amer. Museum Nat. His.	2	A	26.3	2.198	0.0	0.1
SK.	Fairchild	2	A	25.3	2.114	0.0	0.0
580	Nat. Hist.	2	A	26.5	2.215	0.0	0.1
582	Nat. Hist.	2	A	25.7	2.147	0.0	2.4
624	Nat. Hist.	2	A	20.9	1.747	0.0	0.0
$Bulgar_1$	Milk (fermented)	2	B	13.0	1.087	0.2	0.2
$Bulgar_2$	Milk (fermented)	2	B	14.8	1.237	0.1	0.1
Zoo	Milk (fermented)	2	B	14.4	1.199	0.0	0.0
B.B.	Drug Store	2	A	25.4	2.122	0.0	0.2
Massolin	Lederle	2	A	11.4	0.953	—0.4	0.4
Bulgara	Tablet	2	A	28.0	2.319	0.2	0.2
$Bacilline_2$	Tablet	2	A	24.6	2.056	0.0	0.6

menters forming inactive lactic acid and staining solidly are called Type A, while those bacilli that are less active, forming levorotary acid and exhibiting purplish granules when stained are termed Type B. In the present work only the staining reaction was made use of in separating these two types. Culture "Massolin" showed a lower acid production than any of the Type B strains, though this is the only exception. It will be noticed that all three of the saccharose fermenters are of Type A.

Microscopic observation showed the organisms of this group to be Gram-positive long bacteria. In older cultures Gram-negative forms occur, and in one instance the buds and stems mentioned by White and Avery were found. Branch and string formations were noted, but there were no short streptobacilli in this group.

The bacteria included in Table 2 form both types of colonies. Milk is coagulated in from forty-two hours to six days. The lactic acid production varied from 0.201 per cent. to 1.304 per cent., the maximum acidity here being about one-half as great as the maximum of Group 1. As the table shows, the bacilli were isolated from various sources. Both long and streptobacilli occurred, and with the exception of culture "S 11/24" in maltose and saccharose, and culture "Jam" in saccharose, the acid production was marked. Although the limit for clotting was set at six days most of the strains included in the above table caused solidification of the milk before ninety-six hours. In no instance was much whey expressed from the clot.

TABLE 2.

FERMENTATIVE ACTIVITY OF BACTERIA OF GROUP 2

Culture	Source	Type of Colony (Mercshkowsky)	Type of Bacillus	Total Acidity	Lactic Acid	Maltose	Saccharose
K	Typhoid stool	1	Streptobacillus	12.5	1.045	2.5	3.6
Jam	Saliva	1	Long	9.1	0.753	4.6	0.2
G	Typhoid stool	1	Long	8.5	0.710	8.0	0.2
$_{11}$	Putrefactive stool	1	Long	4.2	0.350	4.1	2.0
$_{2}$a	Normal stool	1	Streptobacillus	12.8	1.070	6.0	5.0
HF$_9$	Typhoid stool	1	Long	8.0	0.669	6.1	1.2
HF$_7$	Typhoid stool	1	Long	5.6	0.468	8.2	5.8
HF$_3$	Typhoid stool	1	Long	4.0	0.334	1.3	1.1
S11/24	Typhoid stool	2	Long	8.6	0.719	1.1	0.2
Boh$_2$	Normal stool	1	Streptobacillus	11.8	0.986	0.8	0.7
Boh	Normal stool	2	Long	4.3	0.361	3.4	2.0
DGC	Typhoid stool	1	Streptobacillus	15.6	1.304	4.1	3.1
[illegible]	Typhoid stool	1	Long	5.8	0.485	5.1	4.0
Alt.	Normal stool	1	Long	6.1	0.510	5.4	3.6
[illegible]$_2$	Fermented milk	1	Long	3.6	0.301	1.9	1.0
Sim	Normal stool	1	Long	10.4	0.869	6.3	1.2
Fly	Intestine of fly	1	Long	2.4	0.201	1.3	1.1
DGC$_2$	Typhoid stool	1	Long	15.7	1.312	5.3	4.1
B	Typhoid stool	1	Streptobacillus	2.0	0.167	4.4	0.7
R$_2$	Normal stool	1	Streptobacillus	11.8	0.953	8.0	6.0
HF$_4$	Typhoid stool	1	Long	8.0	0.134	5.4	1.2

It will be seen on reference to the table that in milk both the most and least active strains are streptobacilli and that these form both types of colonies. Both long and streptobacillus forms showed bipolar staining organisms with Löffler's stain. Although the time of clotting is of some value in differentiating the members of this group from those of Group 1, the essential difference is their active splitting of maltose, and to a less extent that of saccharose.

In this group (Table 3) we have the bacilli that do not clot milk, and the acid production in no instance rises above 2.3 per cent. normal. Both types of bacilli and both types of colonies were present.

The tables show that all of the strains investigated fell naturally into three groups according to their action on milk or maltose broth. The organisms of Table 1 were in some instances pure cultures, in others isolated from lactic acid bacilli tablets or from fermented milks. None of the bacteria isolated from other sources were of this type. The members of Group 2 sometimes showed an acidity in milk nearly

TABLE 3.

FERMENTATIVE ACTIVITY OF BACTERIA OF GROUP 3

Culture	Source	Type of Colony (Mereshkowsky)	Type of Bacillus	Total Acidity Percentage of Normal Acid Six Days in Milk	Percentage of Fixed Acid in Terms of Lactic Acid	Percentage of Normal Acid in Maltose Broth, Five Days	Percentage of Normal Acid in Saccharose Broth, Five Days
F	Typhoid stool	1	Long	0.3	0.025	8.8	10.4
S	Putrefactive stool	1	Long	2.3	0.192	1.8	1.8
Kor	Typhoid stool	1	Long	1.3	0.109	6.0	8.0
C	Typhoid stool	1	Streptobacillus	0.3	0.025	9.4	6.6
Milk	Pasteurized milk	1	Long	0.7	0.059	8.8	8.4
D	Normal stool	1	Long	0.6	0.050	7.3	9.3
P	Typhoid stool	1	Long	1.0	0.084	5.4	0.5
HF	Typhoid stool	1	Long	0.2	0.017	6.5	7.7
CF	Typhoid stool	2	Long	1.8	0.151	1.2	1.7
Te	Saliva	1	Long	0.1	0.008	9.2	4.1
Wil	Saliva	1	Streptobacillus	0.7	0.059	9.8	8.5
Rat	Rat feces	1	Long	0.4	0.033	10.3	10.3
CF_2	Typhoid stool	1	Long	0.0	0.0	10.4	8.9
HF_8	Typhoid stool	1	Long	0.8	0.067	2.0	0.2
G_9	Putrefactive stool	2	Long	0.0	0.0	7.3	6.6
Wrgt	Normal stool	1	Long	0.8	0.067	5.1	6.7
$Wrgt_2$	Normal stool	1	Long	1.2	0.101	5.2	6.4
Tas	Normal stool	1	Long	1.2	0.101	8.8	0.0
Bacilline	Tablet	2	Long	0.0	0.0	3.4	4.0
R	Normal stool	1	Long	1.0	0.084	1.8	4.1
O. T.	Normal stool	2	Long	2.2	0.184	1.4	1.0

as great as that of some of the organisms of Group 1, but they were sharply set off from it by their action on maltose. In Group 3 milk is not coagulated, and the action on both maltose and saccharose on the whole is greater here than in either of the preceding groups.

It would appear that the bacillus bulgaricus[17] forms but one type of colony while the bacteria of the "acidophilus" variety form two. On what the colony variation in the latter depends the writer does not attempt to say, but neither the opinion of Sandberg,[18] that it is due

17. The writer is indebted to Prof. C. E. A. Winslow, American Museum of Natural History, New York City, for the pure cultures of the bacillus bulgaricus.

18. *Ztschr. f. klin. Med. Berl.*, 1903, 51, p. 80.

to increased acid production, nor that of White and Avery,[19] that it is dependent on the composition and consistency of the medium, seems to explain the variation.

Tested in milk at room temperature, members of these groups showed widely varying degrees of activity. Only one culture in Group 1 clotted the milk in fourteen days. Group 2 showed greater activity, some strains clotting in six or seven days. In Group 3 culture "Milk" clotted in fourteen days though it did not do so at 38 C.

Tables 4, 5 and 6 show the effect of these bacteria on a series of five carbohydrates after five days at 37 C. For convenience the columns showing the action on maltose and saccharose are reprinted in these tables.

TABLE 4.

THE ACTION OF GROUP 1 ON CERTAIN CARBOHYDRATES

Culture	Percentage of Normal Acid in Dextrose Broth	Percentage of Normal Acid in Lactose Broth	Percentage of Normal Acid in Saccharose Broth	Percentage of Normal Acid in Maltose Broth	Percentage of Normal Acid in Levulose Broth	Percentage of Normal Acid in Mannite Broth
Museum	5.7	5.9	0.1	0.0	[illegible]	[illegible]
SK	16.0	13.9	0.0	0.0	2.4	0.2
580	7.4	7.5	0.1	0.0	2.3	—0.2
582	5.9	3.1	2.4	0.0	[illegible]	0.2
624	5.4	0.9	0.0	0.0	0.7	—0.2
Bulgara	0.4	3.6	0.2	0.2	[illegible]	0.0
Bulgar$_2$	1.3	0.8	0.0	0.1	[illegible]	0.2
Zoo	4.5	2.4	0.0	0.0	[illegible]	0.2
B. B.	2.1	2.0	0.2	0.0	0.0	0.4
Massolin	8.6	5.8	0.4	0.4	0.2	—0.2
Bulgara	1.8	1.6	0.2	0.2	0.2	0.3
Bacilline$_2$	8.4	3.4	0.6	0.0	[illegible]	—

It is evident from this table that these strains have a decided preference for dextrose and lactose; in only two or possibly three instances was saccharose attacked. Seven of the twelve strains did not attack levulose, and mannite showed only a very slight fermentation. In those carbohydrates that were but slightly acted on there were occasional instances in which there was a reduction in the acidity. Duplicate tests confirmed this and the same effect occurred elsewhere as can be seen by reference to the tables. Table 5 shows the results with the organisms of Group 2 on the same sugars.

19. *Centralbl. f. Bacteriol.*, 1910, 53, p. 181.

A comparison of this table with Table 2 shows that the acid production in glucose broth in most instances compares very well with that in milk. Lactose, saccharose, and levulose are utilized by all or nearly all of its members, and the differential value of maltose is strongly brought out. Culture "S $11/_{24}$" is the only organism whose place in this group may be questioned. It was placed here because of its rather slow coagulation of milk—seventy-two hours at 38 C. It is interesting to note that all of the mannite fermenters fall within this group, the sole exception being culture "Wil" in Group 3.

TABLE 5.

THE ACTION OF GROUP 2 ON CERTAIN CARBOHYDRATES

Culture	Percentage of Normal Acid in Dextrose Broth	Percentage of Normal Acid in Lactose Broth	Percentage of Normal Acid in Saccharose Broth	Percentage of Normal Acid in Maltose Broth	Percentage of Normal Acid in Levulose Broth	Percentage of Normal Acid in Mannite Broth
K	12.2	8.4	3.6	2.5	11.3	4.1
Jam	7.0	6.7	0.2	4.6	10.1	1.7
G	7.7	6.7	10.5	8.0	10.0	1.9
G_1	0.5	1.4	2.0	4.3	8.2	1.8
R_2a	7.1	5.0	6.0	3.4	9.1	3.8
HF_6	3.3	1.2	1.2	4.2	7.1	1.4
HF_7	7.5	5.8	5.8	8.2	8.5	0.0
HF_4	4.4	2.4	0.2	3.4	3.3	2.0
HF_5	1.0	0.2	1.2	1.2	0.7	0.6
$S11_{24}$	1.4	1.2	0.2	0.3	0.6	1.0
Boh_1	9.0	8.0	0.7	0.8	10.0	2.8
Boh	2.8	1.8	2.0	1.1	1.2	0.0
DGC	10.0	8.4	3.1	3.5	10.4	2.0
L	5.8	4.0	4.0	5.1	5.8	2.6
Mt.	3.9	7.7	3.6	8.4	9.7	0.8
Z_2	0.8	3.2	1.9	1.9	0.6	0.0
Sim	8.2	8.0	1.2	0.6	11.0	1.8
Fly	2.4	1.6	1.7	1.2	1.4	0.0
$D.GC_2$	7.9	4.6	4.8	6.0	9.6	2.6
B	8.6	4.4	0.7	4.4	8.4	1.4
R_2	7.3	4.4	6.0	4.9	9.1	3.8

Table 6 contains the members of Group 3. Although thirteen out of twenty of these strains showed marked acid production in lactose broth the acid formed in milk was very much less.

From the experiments detailed it seems that the bacilli of the bulgaricus type differ in some important essentials from those usually included under the term "acidophilus." To the writer it appears probable that the bacillus bulgaricus is a milk bacillus, and that its appearance in the intestine practically never occurs unless it has been ingested in enormous numbers. Bacteria of the acidophilic type, on

the other hand, are normal inhabitants of the digestive tube. The question of the survival of the bacillus bulgaricus in the intestine in the light of its identification by means of the maltose reaction will be considered elsewhere.

In addition to the media mentioned above, cultivation of these bacteria was attempted in sugar-free broth and agar. With the exception of a very faint growth in the case of culture "Kor" of Group 3 none of the strains grew in sugar-free broth. The acidity of this broth was + 2.8 to phenolphthalein. On sugar-free agar, however,

TABLE 6.

THE ACTION OF GROUP 1 ON CERTAIN CARBOHYDRATES

Culture	Percentage of Normal Acid in Dextrose Broth	Percentage of Normal Acid in Lactose Broth	Percentage of Normal Acid in Saccharose Broth	Percentage of Normal Acid in Maltose Broth	Percentage of Normal Acid in Levulose Broth	Percentage of Normal Acid in Galactose Broth
F	10.4	9.8	10.4	8.8	12.5	[illegible]
S	3.2	1.5	1.8	1.[illegible]	[illegible]	[illegible]
Kor	4.6	7.9	8.0	6.0	[illegible]	0.0
C	3.4	11.5	6.6	[illegible]	3.1	0.1
Milk	4.1	0.9	8.4	8.8	7.9	0.1
D	6.6	4.6	9.2	7.3	8.4	[illegible]
P	4.0	3.2	0.5	5.4	7.2	0.4
HF	7.8	0.2	7.7	[illegible]	8.0	0.0
CF	1.0	1.0	1.7	1.2	4.4	0.0
Te	4.4	5.7	4.1	9.2	[illegible]	0.2
Wil	6.0	6.3	8.5	9.8	15.0	3.2
Rat	10.1	6.6	10.3	10.3	6.1	0.1
CF_2	11.0	11.0	8.9	10.4	2.8	0.0
[illegible]	7.9	6.8	6.6	7.3	1.1	[illegible]
Wrgt	5.1	4.0	[illegible]	5.1	6.2	0.2
$Wrgt_2$	0.9	5.6	2.4	[illegible]	[illegible]	0.0
Bacilline	0.6	1.4	4.0	[illegible]	2.6	0.3
O. T.	0.4	1.0	1.0	1.4	1.9	0.2
Tas	8.5	6.4	[illegible]	1.8	1.5	0.4
Ri	1.8	0.6	4.1	[illegible]	2.0	[illegible]

different results were obtained. Although after seventy-two hours the members of Group 1 showed no growth, those of Groups 2 and 3 showed fair development in every instance, though the colonies were small. This agar had a reaction of + 1.

In order to study the production of cloudiness in agar, puncture cultures were made in oleate agar. This clouding was not a constantly occurring characteristic, though it appeared in all three groups. In Group 1, in those instances in which it did happen, the cloudiness appeared after seventy-two hours' incubation in the lower part of the tube and in the region of the puncture, spreading outward and upward

until the end of six days when the agar was uniformly clouded. Among the members of Groups 2 and 3 the cloudiness started earlier and spread evenly. Although a good growth was obtained in every instance, some strains showed not the slightest cloudiness even after six days at 37 C.

In addition the effect of these bacteria on raffinose was studied. This sugar was attacked in a greater or less degree by all of the bacilli of Groups 2 and 3 but those of Group 1 did not act on it.

CONCLUSIONS

From the facts brought out in this investigation the writer is of the opinion that there are three varieties of bacilli in the division of acid-resisting bacteria, at least two of which are of constant occurrence among the fecal organisms:

Variety A, which clots milk but has no action on maltose; Variety B, which clots milk and ferments maltose; and Variety C, which ferments maltose but does not clot milk.

Bacilli of the type of the bacillus bulgaricus may be cultivated in unneutralized meat-peptone broth containing a suitable carbohydrate.

In broth the bacillus bulgaricus does not ferment maltose, and may be differentiated from the other acid-resisting organisms by this characteristic.

The writer acknowledges his indebtedness to Dr. J. C. Torrey for valuable suggestions.

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[From the Department of Experimental Pathology in the Medical College of Cornell University, New York City.]

A study of the Anticomplementary action of Yeast, of Certain Bacteria and of Cobra-Venom.

By **Arthur F. Coca.**

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The anticomplementary influence of yeast was first observed by v. Dungern[1]) and his observation was confirmed by Ehrlich and Sachs[2]) who later[3]) employed this method of inactivation in the demonstration of "Complementoid".

The influence of yeast upon complement has been assumed to consist in an absorption of that reagent from the serum, though heretofore, the possibility of an inhibitory influence on the part of soluble substances derived from the yeast has not been experimentally excluded.

The purpose of the present study was to determine whether the inactivation of serum with yeast is due to a process of absorption and, if so, which of the known component parts of that reagent are removed by the yeast.

Technical.

The yeast used in the following experiments was a pure 48 hour saccharose-wort-agar culture of baker's yeast which had been twice washed with 20 volumes of a 0.45 % NaCl solution by rapid centrifugation and suspended, in the first experiments, upon the basis of the volume of moist sediment, in ten volumes of 9 % NaCl solution. In the later experiments a 20 % suspension of the yeast cells was used.

The complement-bearing serum was that of the guinea-pig and of the dog, obtained by centrifugation of the freshly obtained defibrinated blood.

The indicator of the complementary action of the guinea-pig's serum consisted in a 2½ % or a 5 % suspension (based on the volume of corpuscular sediment) of sheep's blood with which had been mixed five minimal hemolytic doses of an anti-sheep's corpuscle rabbit's serum. Of these

1) Münch. med. Wochenschr., 1900, No. 20.
2) Berl. klin. Wochenschr., 1902, No. 14/15.
3) Berl. klin. Wochenschr., 1902, No. 21.

suspensions of sensitized sheep's corpuscles the unit used was $^1/_{10}$ or $^1/_{20}$ c. c. respectively.

The complementary function of the dog's serum was tested with a $2^1/_2$ % suspension of washed guinea-pig's blood corpuscles. The guinea-pig's corpuscles were sensitized for the action of dog's complement by the natural sensitizing substances of the dog's serum, these being usually present in amount sufficient for the purpose in the various mixtures used.

In order to determine whether the anticomplementary influence of yeast is due to a process of absorption or to soluble inhibiting substances, one cubic centimeter of guinea-pigs's serum was mixed respectively with 2.0 c. c. of the yeast suspension and with 2.0 c. c. of the clear supernatant fluid that was obtained after rapid centrifugation of the yeast suspension. The yeast suspension had been standing at room temperature for about two hours.

After these mixtures had stood for one hour at room temperature [1]), the one that contained the yeast bodies was centrifugated and the supernatant fluid "A" was compared with the other mixture "B" as to its complement content. The result of this comparison is shown in Table I.

Table I.

Amount of the serum mixtures	Hemolysis of $^1/_{20}$ c. c. of a 5% suspension of sensitized sheep's corpuscular sediment	
c. c.	A.	B
$^2/_{10}$	0	c.
$^1/_{10}$	0	c.
$^1/_{20}$	0	c.
$^1/_{40}$	—	c.
$^1/_{80}$	—	c.
$^1/_{160}$	—	n. c.

Note. In this and the succeeding tables the following abbreviations are used: c. complete hemolysis; n. c. nearly complete hem.; v. st. very strong hem.; st. strong hem.; m. moderate hem.; sl. slight hem.; 0 no hem.

The result of the foregoing experiment indicates that the anticomplementary influence of yeast is due to a process of absorption. This experiment was repeated, one volume of serum being mixed with two volumes of the decanted yeast fluid and the same results were obtained. There remained

1) The inactivation of serum with yeast takes place more rapidly at 37° C.

to be determined, therefore, which of the constituents of the serum that together perform the complementary function, are taken up by the yeast bodies.

Before the investigation of this question could be undertaken it was necessary to obtain preparations of the "mid-piece" and "end-piece" of complement. For this purpose the simple carbon-dioxide method of Liefmann was employed.

Carbon dioxide gas was passed for 5 to 10 minutes through 10 c. c. of a 1—5 dilution of guinea-pig's serum in distilled water, and the resulting precipitate was then separated from the fluid by rapid centrifugation and decantation. The clear decanted fluid contained the "end-piece" of complement and will be designated the end-piece solution. The entire end piece solution was rendered isotonic by the addition to it of $^1/_{10}$ of its volume of a 9.0 % NaCl solution. The tubes in which the precipitate was thus collected were rinsed out with distilled water and the precipitate was rubbed up by means of a glass rod with 4.0 c. c. of physiological saline solution, whereby the greater part of the precipitate went into an opalescent solution. This fluid, which contained the "mid-piece" of complement, will be designated the mid-piece solution.

By itself, 0.4 c. c. of the end-piece solution was able to dissolve the unit of sensitized sheep's blood corpuscles ($^1/_{20}$ c. c. of a 5 % suspension) in one preparation slightly, and in a second preparation not at all. Combined with $^1/_{20}$ c. c. of the mid-piece solution, — c. c. of the end-piece solution caused complete hemolysis. $^1/_{40}$ c. c. nearly complete, and — slight hemolysis of the unit of sensitized sheep's blood.

Accepting $^1/_{30}$ c. c. as the smallest completely hemolytic amount of the end-piece solution, this amount is found to correspond with $^1/_{165}$ c. c. of the undiluted serum (i. e. $^1/_{30} \times ^1/_5 \times ^{10}/_{11}$).

By itself, 0.2 c. c. of the mid-piece solution of both preparations was entirely lacking in hemolytic power when mixed with the unit of sensitized sheep's blood; but when combined with either 0.2 c. c. or 0.1 c. c. of the end-piece solution, $^1/_{80}$ c. c. of the mid-piece solution produced complete hemolysis and $^1/_{160}$ c. c. strong hemolysis of the unit of sheep's blood. The entire carbon dioxide precipitate from 2.0 c. c. of serum having been taken up in 4.0 c. c. of salt solution in the preparation of the mid-piece solution, the smallest completely hemolytic amount of the mid-piece solution ($^1/_{80}$ c. c.) was derived accordingly from $^1/_{40}$ c. c. of the undiluted guinea-pig's serum. From these titrations, the results of which were identical with preparations from sera of two different guinea-pigs, it is seen that the smallest completely hemolytic amounts of end-piece and mid-piece were derived from the same quantity of the untreated serum, i. e. $^1/_{165}$ c. c. This quantity is five times the amount of the untreated serum ($^1/_{30}$ c. c.) that could completely hemolyse the unit of sensitized blood corpuscles, which indicates a corresponding reduction in the general complementary power, due to the process of separation of these two component parts.

With the aid of the end-piece and mid-piece solutions it was possible to determine which, if either, of those two component parts was removed from the guinea-pig's serum by the yeast.

The guinea-pig's serum that had been in contact with the yeast was combined in different amounts with 0.2 c. c. of the end-piece solution and to each of these mixtures was added $^1/_{10}$ c. c. of a $2^1/_2$ % suspension of sensitized sheep's blood. After the mixtures had stood for one hour at 37° C complete hemolysis had taken place in the tubes containing $^1/_{20}$, $^1/_{40}$, $^1/_{80}$, $^1/_{160}$ and $^1/_{320}$ c. c. of the "yeast serum", and with $^1/_{640}$ c. c. the solution of the corpuscles was nearly complete. The result of this test shows that the mid-piece was amply represented in the "yeast serum".

A similar series of mixtures was made, also, with $^1/_{20}$ c. c. of the mid-piece solution, and with this fraction of complement, also, complete hemolysis was produced by $^1/_{20}$ c. c. and slight hemolysis by $^1/_{40}$ c. c. of the "yeast serum". The "yeast serum", therefore, contained, also, end-piece.

The experiments just described show that notwithstanding the presence in the "yeast serum" of both the end-piece and mid-piece of complement, not the least hemolysis was produced by 0.2 c. c. of the "yeast serum" a quantity that demonstrably contained at least 4 minimal hemolytic amounts of the former element and over 60 such quantites of the latter. By these results, therefore, the conclusion seemed forced that in the serum that had been in contact with the yeast cells a third component of complement was lacking.

Upon the basis of the studies carried out in the laboratory of H. Sachs by Sachs and Omorokow[1]), H. Ritz[2]), Husler[3]) and Jonas[4]) on the influence of cobra venom upon complement, the existence of a third component of complement already has been assumed. The obserations that support the assumption of a third component are the following:

1) Zeitschr. f. Immunitätsf., Bd. 11, 1911.
2) Zeitschr. f. Immunitätsf., Bd. 13, 1912.
3) Zeitschr. f. Immunitätsf., Bd. 15, 1912.
4) Zeitschr. f. Immunitätsf., Bd. 17, 1913.

1) Serum that has been inactived with cobra venom can be reactivated with the addition of the end-piece or of the mid-piece of complement, which shows that in the cobra-serum both of these components are present.

2) Serum that has been inactivated with cobra venom can be reactivated with serum that has been heated for half hour at 54° C, or with either end-piece or mid-piece solution after the respective function of these preparations has been completely destroyed by heating them for $^1/_2$ hour at 54° C.

Liefmann[1]) while admitting that these facts indicate the assumption of a third component, considers that they are insufficient fully to establish it. He says, „Man darf die Möglichkeit nicht außer Acht lassen, daß Schädigungen des Komplementes, wie z. B., die durch Cobragift, nicht in einer Zerstörung eines Komplementteiles begründet sind, sondern nur das Milieu, in dem die Komplementwirkung vor sich geht, verändern. — Man kann auch daran denken, daß das Kobragift vielleicht durch einen Hemmungskörper die Wirksamkeit des Komplementes aufhebt. Diese Hemmung müßte dann durch auf 54° erwärmtes Serum beseitigt werden".

This objection cannot well be made in the case of the yeast-serum, since it is probable that the anticomplementary action of the yeast bodies is due to a process of absorption.

We have seen that the cobra-serum and yeast-serum preparations are similar inasmuch as both contain the end-piece and mid-piece of complement. A further similarity of these preparations was found in the fact that the yeast-serum, like the cobra-serum, can be reactivated by the addition of small quantities of serum that has been rendered inactive with heat.

In the experiment directed to the reactivation with heated serum, the results of which are shown in table II, the yeast-sera were prepared by mixing 5 c. c. of serum with $2^1/_2$ c. c. of a 20% suspension of yeast-cells und centrifugating the mixtures after they had stood at room temperature — 20° C — for one hour during which time they had been moderately shaken three or four times.

1) Jahresbericht über die Ergebnisse der Immunitätsf., Bd. 8, 1. Abt., p. 145.

Table II.

Amount of dog's yeast-serum c. c.		Inactivated serum of guinea-pig							Inactivated serum of dog							
		$^2/_{10}$	$^1/_{10}$	$^1/_{20}$	$^1/_{40}$	$^1/_{80}$	$^1/_{160}$	$^1/_{320}$	$^2/_{10}$	$^1/_{10}$	$^1/_{20}$	$^1/_{40}$	$^1/_{80}$	$^1/_{160}$	$^1/_{320}$	0
$^2/_{10}$	S [1]	.	—	—	—	—	—	.	.	—	—	—	—	—	.	0
	G [2]	.	c.	c.	c.	c.	st.	.	.	c.	c.	c.	c.	st.	.	0, ag. [3]
$^1/_{10}$	S	.	—	—	—	—	—	.	.	—	—	—	—	—	.	.
	G	.	c.	c.	c.	c.	st.	.	.	c.	c.	c.	c.	st.	.	0, ag.
$^1/_{20}$	S	.	—	c.	c.	—	—	.	.	—	—	—	—	—	.	.
	G	.	c.	c.	c.	c.	st.	.	.	c.	c.	c.	st.	m.	.	.
$^1/_{40}$	S	.	—	0	0	—	—	.	.	.	—	.	.	.	.	.
	G	.	sl.	sl.	—	—	—	.	.	.	c.	.	.	.	.	.
$^1/_{80}$	S	.	.	.	.	.	.	.	.	.	—	.	.	.	.	.
	G	.	.	.	.	.	.	.	.	.	st.	.	.	.	.	.
$^1/_{160}$	S	.	.	.	.	.	.	.	.	.	—	.	.	.	.	.
	G	.	.	.	.	.	.	.	.	.	m.	.	.	.	.	.

Amount of guinea-pigs yeast-serum c. c.		Inactivated serum of guinea-pig							Inactivated serum of dog							
		$^2/_{10}$	$^1/_{10}$	$^1/_{20}$	$^1/_{40}$	$^1/_{80}$	$^1/_{160}$	$^1/_{320}$	$^2/_{10}$	$^1/_{10}$	$^1/_{20}$	$^1/_{40}$	$^1/_{80}$	$^1/_{160}$	$^1/_{320}$	0
$^2/_{10}$	S	.	.	.	.	.	.	.	.	.	c.	.	.	.	.	0
$^1/_{10}$	S	.	c.	c.	.	.	.	.	.	.	c.	c.	c.	.	.	0
$^1/_{20}$	S	.	st.	c.	c.	st.	m.	sl.	.	m.	st.	c.	c.	c.	m.	.
$^1/_{40}$	S	.	st.	c.	.	.	.	.	.	.	0	c.	st.	.	.	.
$^1/_{80}$	S	.	m.	c.	.	.	.	.	.	.	0	sl.	sl.	.	.	.
$^1/_{160}$	S	.	sl.	m.	.	.	.	.	.	.	0	0	.	.	.	.
0	S	0	0	.	.	.	.	.	m.	sl.	0	.	.	.	.	.
	G	—	—	.	.	.	.	.	m.	sl.	0	.	.	.	.	.

1) S = 0.1 c. c. $2^1/_2$ % sensitized sheep's corpuscles.
2) G = 0.1 c. c. $2^1/_2$ % guinea-pig's corpuscles.
3) ag. = agglutination of the corpuscles.

The „inactivated" guinea-pig's serum had been heated for ½ hour at 54½° C, the inactivated dog's serum had been heated for ½ hr. at 51° C. Different quantities of the two yeast-sera were combined with different quantities of the two heated sera, the reactivation of the yeast-serum of the dog being tested for the greater part with guinea-pig's corpuscles (G), whereas the reactivation of the yeast-serum of the guinea-pig was tested only with sensitized sheep's corpuscles (S).

The degree of hemolysis produced in the unit of corpuscles (1/10 c. c. of a 2½ % suspension of corpuscular sediment) by the different combinations of yeast-serum and inactivated serum is indicated at the intersection of the respective columns.

The result of this experiment may be summarized as follows:

1) Both the dog's-serum and the guinea-pig's serum that had been in contact with the yeast had completely lost their general complementary power [1]) (right-hand marginal column).

2) The complementary function of 1/10 c. c. of dog's yeast-serum was completely restored by the same minimal quantity of guinea-pig's inactivated serum or of dog's inactivated serum, viz: 1/60 c. c.

3) The complete reactivation of 1/20 c. c. of guinea-pig's yeast-serum was accomplished with less of the dog's inactivated serum than of the inactivated serum of the guinea-pig. This result may have been due to the influence of the natural antisheep's corpuscle amboceptor in the dog's serum which was able, in the unheated condition, completely to dissolve the unit of unsensitized sheep's corpuscles in a quantity of 1/200 c. c.

4) Both the guinea-pig's inactivated serum and the dog's inactivated serum exert an anticomplementary influence when combined with guinea-pig's yeast-serum. This influence is apparent where the larger amounts of the heated sera — 1/10 c. c. or 1/20 c. c. — are mixed with the smaller quantities — 1/20 c. c. or less — of the yeast serum, and it is stronger in the dog's heated serum than in the guinea-pig's heated serum. The anticomplementary action of the heated sera was

1) Complete hemolysis of the unit of guinea-pig's corpuscles was produced by 1/200 c. c. of the normal dog's serum, and the unit of sensitized sheep's corpuscles was completely dissolved by 1/200 c. c. of normal guinea-pig's serum.

not seen where these were combined with the dog's yeast-serum.

5) It is seen that when mixed with constant quantities of the heated sera a larger amount of the dog's yeast-serum is required to cause hemolysis when it is combined with inactivated guinea-pig's serum than when mixed with inactivated dog's serum. This advantage on the part of the heated dog's serum may be due to its large content in natural amboceptor against both guinea-pig's corpuscles and sheep's corpuscles.

All of the experiments that we have described point to an identity of influence on the part of cobra venom and yeast cells upon the complementary system. Both of these materials leave the mid-piece and the end-piece of complement apparently unaffected but deprive the serum of the function of a third component of complement, which is present in active condition in heated serum.

A further comparative examination of the two inactivation processes was undertaken, on the one hand, by means of a quantitative study of the relationships of yeast-serum and cobra-serum toward heated serum and on the other hand, by combining the yeast-serum and cobra-serum preparations in different proportions.

The investigation in this direction was disappointing, inasmuch as totally different results were obtained with the sera of different guinea-pigs.

The sera of eleven guinea-pigs were examined. It will be convenient first to describe in detail the plan of study of one of these sera and to discuss the results of the experiments in turn.

For the following experiments the guinea-pig's yeast-serum was prepared by mixing 4.0 c. c. of fresh guinea-pig's serum with the sediment (obtained by centrifugation) of 2.0 c. c. of a 20% suspension of yeast cells, and allowing this mixture to stand for one hour at room temperature. The yeast was then removed by centrifugation and decantation. Mixed with the „unit"[1]) of sensitized sheep's blood corpuscles this serum preparation caused no hemolysis in a quantity of $^2/_{10}$ c. c. or less.

1) The unit of corpucles for this entire experiment was $^1/_{20}$ c. c. of a 5% suspension of the corpuscular sediment of washed sheep's blood to which had been added five hemolytic doses of amboceptor (rabbit).

For the inactivation of the guinea-pig's serum with cobra-venom the proportions employed by Omorokow were used. A mixture of 4.0 c. c. of guinea-pig's serum and 1.6 c. c. of a $^1/_8$ % solution of cobra-venom [1]) was kept at a temperature of 37° C and at intervals the mixture was tested as to its complementary activity. At the end of $1^1/_4$ hours the unit of sensitized shee'ps corpuscles could be completely hemolysed with $^1/_{10}$ c. c. of the mixture. At the end of two hours $^2/_{10}$ c. c. was still table to cause complete hemolysis, and $^1/_{10}$ c. c. strong hemolysis when mixed with the unit of sheep's blood, and at the end of $4^1/_2$ hrs. no hemolysis could be produced with $^1/_{10}$ c. c. and only a trace of hemolysis with $^2/_{10}$ c. c. of the serum-venom mixture. In the succeeding experiments the cobra-serum used had been kept at 37° C for 5 hours and thereafter at room temperature without further dilution excepting as was required for the performance of the different tests.

In a preliminary experiment the reactivation, according to Ritz, with guinea-pig's serum that had been heated for $^1/_2$ hour at 54° C was carried out with $^1/_{10}$ c. c. and $^1/_{80}$ c. c. of the yeast-serum and of the cobra-serum [2]). The results of this experiment are shown in table III.

Table III.

Amount of 54°-serum	Hemolysis of $^1/_{20}$ c. c. of 5 % sensitized sheep's corpuscles produced by constant quantities of yeast-serum or cobra-serum combined with diminishing amounts of heated serum			
	yeast-serum		cobra-serum	
c. c.	$^1/_{10}$ c. c.	$^1/_{80}$ c. c.	$^1/_{10}$ c. c.	$^1/_{80}$ c. c.
$^1/_{40}$	c.	c.	—	—
$^1/_{80}$	c.	—	n. c.	c.
$^1/_{160}$	n. c.	c.	m.	—
$^1/_{320}$	m.	c.	sl.	—
$^1/_{640}$	0	—	0	n. c.
$^1/_{1280}$	0	c.	0	sl.
$^1/_{2560}$	0	m.	—	0
$^1/_{5120}$	0	sl.	—	0

The experiment confirms the observation of Ritz that serum that has been inactivated with cobra-venom can be reactivated with serum that has been inactivated with heat and shows again that the same thing is true of yeast-serum.

1) For a generous quantity of cobra-venom I am greatly indebted to Major W. Glen Liston, Bombay, Bacteriological Laboratory.

2) In all such comparative tests the quantity of cobra-serum and yeast-serum actually used was such as contained the indicated quantity of undiluted guinea-pig's serum.

It is seen, furthermore, that more of the heated serum was required for the reactivation of both the yeast serum and the cobra-serum when these were used in larger amounts, than when they were used in smaller amounts. It appears, also, that, in this test, both the larger and smaller amounts of the cobra-serum required more of the heated serum for reactivation than did the corresponding quantities of the yeast-serum.

A corresponding difference in the quantitative relationships of the yeast-serum and cobra-serum preparations toward heated serum was also observed when constant quantities of the heated serum were combined with descending amounts of yeast-serum and cobra-serum respectively. The results of this experiment are shown in table IV.

Table IV.

Amount of cobra-serum or yeast-serum	Hemolysis of $^1/_{20}$ c. c. of 5 % sensitized sheep's corpuscles produced by constant quantities of heated serum combined with diminishing quantities of cobra-serum or of yeast-serum					
	54 °-serum $^1/_{20}$ c. c.		54 °-serum $^1/_{160}$ c. c.		54 °-serum $^1/_{320}$ c. c.	
c. c.	cobra-ser.	yeast-ser.	cobra-ser.	yeast-ser.	cobra-ser.	yeast-ser.
$^1/_{80}$	c.	c.	c.	c.	c.	c.
$^1/_{160}$	c.	c.	c.	c.	c.	c.
$^1/_{320}$	c.	c.	c.	c.	c.	c.
$^1/_{640}$	sl.	sl.	c.	c.	n. c.	c.
$^1/_{1280}$	0	0	v. st.	n. c.	m.	v. st.
$^1/_{2560}$	0	0	sl.	sl.	0	0

It is seen that where the larger amount ($^1/_{10}$ c. c.) of the heated serum was used, the same quantity ($^1/_{320}$ c. c.) of cobra-serum or of yeast-serum was needed to supply a completely hemolytic amount of complementary substances, and furthermore, that in both instances this quantity was greater than was required to supplement the smaller amounts of the heated serum. Where the smaller quantities of the heated serum were used, less of the yeast-serum was required to cause complete hemolysis than of the cobra-serum.

The combination of yeast-serum and cobra-serum was made in four series, in two of which constant quantities of yeast-serum were mixed with descending quantities of cobra-serum, while in the other two series constant quantities of

cobra-serum were mixed with descending amounts of yeast-serum.

The results of this experiment are shown in table V.

Table V.

Hemolysis of $^1/_{20}$ c. c. of 5 % sensitized sheep's corpuscles by different quantitative combinations of cobra-serum and yeast-serum.

	yeast-serum $^1/_{10}$	yeast-serum $^1/_{40}$	cobra-serum $^1/_{10}$	cobra-serum $^1/_{40}$
c. c.	cobra-serum		yeast-serum	
$^1/_{10}$	m.	trace	m.	st.
$^1/_{20}$	st.	trace	sl.	m.
$^1/_{40}$	st.	sl.	trace	sl.
$^1/_{80}$	v. st.	m.	0	sl.
$^1/_{160}$	v. st.	st.	—	trace
$^1/_{320}$	v. st.	v. st.	—	0
$^1/_{640}$	v. st.	—	—	—

The tests described in table V were repeated with the same preparations on the following morning and identical results were obtained.

Table VI.

Serum No.		Amounts of yeast-serum or of cobra-serum	Hemolysis of 0.1 c. c. of 2½ % sensitized sheep's corpuscles with constant quantities of yeast-serum or of cobra-serum combined with descending quantities of the corresponding serum heated for ½ hr. at 54° C			
		c. c.	$^1/_{160}$	$^1/_{320}$	$^1/_{640}$	$^1/_{128}$
1	y.[1])	$^1/_{160}$	c.	c.	st.	0
	co.	$^1/_{160}$	c.	st.	sl.	0
2	y.	$^1/_{160}$	c.	st.	0	0
	co.	$^1/_{160}$	c.	st.	0	0
3	y.	$^1/_{160}$	c.	st.	sl.	0
	co.	$^1/_{160}$	c.	st.	sl.	0
4	y.	$^1/_{160}$	c	c.	n. c.	sl.
	co.	$^1/_{160}$	c.	c.	mod.	0
5	y.	$^1/_{160}$	c.	c.	st.	tr.
	co.	$^1/_{160}$	c.	n. c.	sl.	0
6	y.	$^1/_{160}$	c.	c.	st.	tr.
	co.	$^1/_{160}$	c.	st.	sl.	0
7	y.	$^1/_{80}$	c.	c.	n. c.	m.
	co.	$^1/_{80}$	c.	c.	c.	st.
8	y.	$^1/_{80}$	c.	c.	c.	st.
	co.	$^1/_{80}$	c.	c.	c.	sl.

1) y. = yeast-serum; co. = cobra-serum.

Eight other guinea-pig sera were examined according to the plan of table III columns 2 and 3. The results of these examinations are given in table VI. A constant small quantity of yeast-serum or cobra-serum was combined with descending amounts of the corresponding inactivated serum. In every instance the cobra-serum preparation was used after 3 hrs. incubation at 37° C. None of the cobra-serum preparations was at all hemolytic in a quantity of $^2/_{10}$ c. c. when mixed with the unit of sensitized sheep's corpuscles.

Table VII.

Serum No.	In each tube yeast-serum	Hemolysis of $^1/_{10}$ c. c. of $2^1/_2$ % sensitized sheep's corpuscles with constant quaantities of yeast-serum combined with descending quantities of the corresponding cobra-serum					
	c. c.	$^1/_{10}$	$^1/_{20}$	$^1/_{40}$	$^1/_{80}$	$^1/_{160}$	$^1/_{320}$
1	$^1/_{20}$	m.	—	st.	m.	sl.	—
2	$^1/_{20}$	0	—	0	0	0	—
3	$^1/_{20}$	sl.	—	m.	sl.	0	—
4	$^1/_{10}$	—	0	st.	st.	m.	m.
5	$^1/_{10}$	—	0	0	0	0	0
6	$^1/_{10}$	—	0	m.	m.	sl.	0
7	$^1/_{10}$	st.	v. st.	c.	c.	n. c.	st.
8	$^1/_{10}$	0	0	0	0	0	—
9	$^1/_{10}$	st.	v. st.	v. st.	v. st.	v. st.	—
10	$^1/_{10}$	sl.	st.	v. st.	v. st.	—	—

It is seen that in cases 1, 4, 5 and 6, as in table III, a larger amount of the inactivated serum had to be combined with the cobra-serum in order to produce complete hemolysis than was necessary with a corresponding amount of the yeast serum. In cases 2, 3 and 8, on the other hand, no difference in this respect was observed, while in case 7 [1]) the relation was found to be just the reverse of that in table III, inasmuch as the yeast-serum required more of the heated serum for the production of complete hemolysis than was necessary with the same quantity of the cobra-serum.

The reactivation of yeast-serum with cobra-serum was attempted with preparations made with ten guinea-pig sera in addition to that used for the test shown in table V. The

1) When this test was repeated on following morning the difference between the two preparations was found to be less but still distinctly present and in the same sense.

results of these reactivation tests are shown in table VII. In every case $^2/_{10}$ c. c. of the cobra-serum preparations was entirely lacking in hemolytic action when mixed with the unit of sensitized sheep's corpuscles. Of the yeast-serum preparations $^2/_{10}$ c. c. in case 1, was strongly hemolytic, $^1/_{10}$ c. c. not at all; $^2/_{10}$ c. c. in cases 3 and 4 was moderately hemolytic, $^1/_{10}$ c. c. not at all; and $^2/_{10}$ c. c. in case 6 was slightly hemolytic, $^1/_{10}$ c. c. not at all. In no other case was $^2/_{10}$ c. c. of the yeast-serum preparations at all hemolytic for sensitized sheep's corpuscles.

It is seen that in case 7 the reactivation of $^1/_{10}$ c. c. of an entirely inactive yeast-serum preparation was complete with relatively small amounts of the inactive cobra-serum. Nearly complete reactivation was obtained, also, in cases 1, 4, 9 and 10. Slight reactivation was seen in cases 3 and 6, and in cases 2, 5 and 8 the cobra-serum preparations lacked completely the power of reactivating the corresponding yeast-serum preparations.

With case 7, $^1/_{10}$ c. c. of the cobra-serum preparation was mixed with descending quantities of the yeast-serum preparation and to these mixtures was added the unit of sensitized sheep's corpuscles. Hemolysis resulted as follows: with $^1/_{10}$ c. c. of yeast-serum strong, with $^1/_{20}$ c. c. slight, with $^1/_{40}$ c. c. a trace and with $^1/_{80}$ c. c. none (compare these results with those of table V, column 3).

In the foregoing experiments it has been shown that by a process of absorption the complementary system of serum is deprived, by yeast cells, of a component that exists in active condition in heated serum as well as in preparations of isolated end-piece and mid-piece of complement. Since it was found that the yeast serum could often be reactivated with small quantities of serum that had been inactivated with cobra-venom, it was assumed that the component of complement that had been removed by the yeast was still present, in active form, in the cobra-serum.

According to this conception the inactivation of serum with cobra-venom is not due to a destruction of the third component of complement but to an inhibition of the function

of that component by some substance in the venom. This inhibiting influence shows itself sometimes in the serum-venom mixtures when relatively small amounts of the third component are added to the inactive cobra-serum in the form of heated serum [1]) (tables III, IV and V).

The fact that a cobra-serum preparation in which the third complementary function has been rendered inactive, may yet be able to supply this function to the corresponding yeast-serum preparation may be explained as follows:

Under circumstances that have not been experimentally studied, the inhibition exercised by the cobra-venom upon the third component of complement is reversed or neutralized when small quantities of the inactive cobra-serum mixture are combined with relatively large amounts of other serum preparations, such as yeast-serum (tables V and VI).

The anticomplementary action of certain bacteria.

The anticomplementary action of bacteria was first observed by von Dungern [2]).

The anticomplementary influence of bacillus prodigiosus has been found by Ritz and Sachs [3]) to resemble that of cobra-venom inasmuch as serum that had been rendered inactive by digestion with these bacteria could be reactivated with either end-piece or mid-piece of complement.

Browning and Mackie [4]) found that staphylococcus aureus did not always inactivate guinea-pig's serum; out of three such experiments two resulted in a partial inactivation and one failed to demonstrate any anticomplementary effect (complete protocols were not published). It appears that reactivation experiments with end-piece and mid piece prepa-

1) The inhibitory property of cobra-serum is very clearly shown in Ritz's experiments (column B, table III, p. 68, and column b, table IV, p. 70).

2) l. c.

3) Centralbl. f. Bakt., I. Abt., Ref., Bd. 50, Beiheft, p. 47. See also Ritz, l. c.

4) Biochem. Zeitschr., Bd. 43, 1912, p. 229. — Journ. of Path. and Bact., Vol. 17, p. 120.

rations were carried out with the sera that had been treated with the bacteria. However, the details of those experiments are lacking in both publications and it is not clearly stated that the reactivation was successful with both mid-piece and end-piece preparations.

In a few experiments we have examined the anticomplementary influence of Sarcina lutea and of Bac. subtilis, these bacteria having been selected because of their relatively large size.

The bacteria were obtained from 48 hr. slanted agar cultures and were washed several times with 0.9 % saline solution.

Guinea-pig's serum that had been in contact with an equal volume of the washed sediment of the subtilis culture were entirely inactive within one hour and could not be reactivated with heated serum that was capable of reactivating both yeast-serum and cobra-serum. It was found, also, that the clear fluid decanted from the bacteria after the last centrifugation was highly anticomplementary.

The fluid decanted from the sarcina after the last centrifugation was not anticomplementary when mixed with an equal volume of guinea-pig's serum. (Table VIII, compare A and B.) Guinea-pig's serum that had been in contact for 1¼ hrs. with an equal volume (a relatively large amount) of the thick sediment of the washed sarcina was not entirely inactive. (Table VIII, C.)

Table VIII.

Amounts of serum mixtures c. c.	Hemolysis of 0.1 c. c. of a 2½ % suspension of sensitized sheep's corpuscles with the following serum mixtures		
	A 50 % guinea-pig's serum	B sarcina-decant guinea-pig's serum equal volume	C sarcina-serum
1/20	—	—	c.
1/50	—	—	sl.
1/100	c.	c.	0
1/200	c.	c.	0
1/400	m.	m.	—
1/800	0	0	—

It was found that the serum that had thus been shown to have lost 90 % of its general complementary activity during its contact with the sarcina bodies could be reactivated with small quantities of serum that had been inactivated by being heated for $^1/_2$ hr. at 54° C. (Table IX.)

Table IX.

Amounts of heated serum c. c.	Hemolysis of $^1/_{10}$ c. c. of a $2^1/_2$ % suspension of sensitized sheep's corpuscles with $^1/_{100}$ c. c. of sarcina-serum combined with heated serum (54° C.)
$^1/_{320}$	complete
$^1/_{640}$	"
$^1/_{1280}$	nearly complete
$^1/_{2560}$	moderate

These observations upon the anticomplementary action of sarcina correspond with those of Ritz and Sachs upon the influence of Bac. prodigiosus referred to above.

As a practical method of obtaining a serum-preparation free of the third component the use of yeast cells is much more convenient than that of bacteria, not only on account of the greater size of the former but because of the completeness of the removal of the component in question by the yeast and the absence of soluble anticomplementary substances in the yeast suspension.

Some experiments bearing upon the nature of the inactivating agent in cobra-venom.

Three experiments were conducted with the purpose of investigating the nature of the substance in cobra-venom which is responsible for the inactivation of serum. We have studied:

first, the influence of heat;

secondly, the influence of sodium chlorid precipitation; and

thirdly, the influence of concentrated sodium chlorid upon the inactivating principle.

The influence of heat upon the inactivating principle of cobra-venom.

It is known that both the hemolysin and the neurotoxin of cobra-venom are relatively very resistent to heat: therefore, it would seem that if either of these agents were directly concerned in the inactivation of serum this property would be preserved in heated cobra-venom. Such, however, is not the case, for we have found that if a 1 % solution of cobra-venom in 0.9 % NaCl solution be heated for ¾ hr. at 72 C., by which treatment the two known toxic agents are not at all affected, the property of the venom solution of inactivating serum is completely lost.

Experiment.

The following mixtures were incubated for 2½ hrs. at 37° C. and their complement content was then tested with ⅒ c. c. of a 2½ % suspension of sensitized sheep's corpuscles.

A	guinea-pig's serum	2 c. c., and
	⅛ % 72° C. cobra-venom	1 „
B	guinea-pig's serum	2 c. c., and
	0.9 % NaCl solution	1 „
C	guinea-pig's serum	2 c. c., and
	⅛ % cobra-serum	1 „

Hemolysis of sensitized sheep's corpuscles after 2 hrs. at 37° C.

	2/10 c. c.	1/10	1/100	1/200	1/400
A	—	—	c.	n. c.	sl.
B	—	—	c.	n. c.	sl.
C	sl.	0	—	—	—

The influence of sodium chloride precipitation upon the inactivating principle of cobra-venom.

We have found that the hemotoxin is practically quantitatively precipitated from a 1 % aqueous solution of cobra-venom by saturating the solution with sodium chloride. The influence of this treatment upon the neurotoxin was not examined. The sodium chlorid precipitate of cobra-venom, when dissolved in a small volume of distilled water is not

coagulable by boiling, in which respect it differs from most sodium chlorid precipitates of animal origin.

The following experiment shows that the agent in cobra-venom that inactivates serum is, also, precipitated by sodium chlorid saturation.

5 c. c. of a 1 % solution of cobra-venom were shaken with about 1.8 gr. of powdered sodium chlorid (Kahlbaum I) until most of the salt was dissolved. The flocculent precipitate that formed was separated from the fluid by centrifugation and decantation, and it was washed once with 6 c. c. of saturated NaCl solution. The entire precipitate was dissolved in 5 c. c. of distilled water and this solution was diluted 1 to 3, also with distilled water. 0.5 c. c. of this diluted solution of the sodium chlorid precipitate was found to inactivate 1.0 c. c. of guinea-pig's serum; after being incubated for $3^1/_2$ hrs. at 37 ° C, $^2/_{10}$ c. c. of this mixture was able to cause only slight hemolysis of $^1/_{10}$ c. c. of a $2^1/_2$ % suspension of sensitized sheep's corpuscles.

The influence of concentrated sodium chlorid upon the inactivating principle of cobra-venom.

A moderate degree of concentration of sodium chlorid markedly inhibits the inactivating power of cobra-venom.

After 4 hrs. incubation at 37 ° C, a mixture of 1.0 c. c. of guinea-pig's serum and 0.5 c. c. of a $^1/_6$ % solution of cobra-venom in a 5 % solution of sodium chlorid had lost only 50 % of its complementary activity.

Zusammenfassung.

1) Die Inaktivierung des Serums durch Hefe beruht wahrscheinlich auf einem Absorptionsvorgang.

2) Ein Serum, das mit Hefe inaktiviert worden ist, enthält das Endstück und das Mittelstück des Komplementes. Einem solchen Serum fehlt ein Bestandteil des Komplementes der im erhitzten Serum noch in aktivem Zustande vorhanden

ist. Dieses fehlende Element ist wahrscheinlich mit der „dritten Komponente" von Sachs, Omorokow und Ritz identisch.

3) Mit einer Anzahl von verschiedenen Meerschweinchenseren konnte Hämolyse der sensibilisierten Hammelblutkörperchen durch Mischungen von Cobraserum mit Hefeserum herbeigeführt werden, obgleich diese Präparate für sich allein gar nicht lösten. Mit anderen Meerschweinchenseren hergestellte derartige Mischungen waren aber ganz wirkungslos.

4) Die antikomplementäre Wirkung der Bakterien kann bei den verschiedenen Arten dieser Organismen verschieden sein. Einige Bakterien (Bac. subtilis) produzieren lösliche antikomplementäre Stoffe, währenddem von anderen (Sarcina lutea) derartige Substanzen nicht gebildet werden.

Die Inaktivierung des Serums durch Sarcina lutea beruht auf einem Absorptionsvorgang. In diesem Falle wurde es gefunden, in Uebereinstimmung mit den früheren Befunden von Ritz und Sachs, daß dem teilweise inaktivem Serum eine dritte Komponente des Komplementes fehlt, die im erhitzten Serum in aktivem Zustande vorhanden ist.

5) Durch das Erhitzen einer 1-proz. Lösung des Cobragifts ($^3/_4$ Stunde bei 72° C) wird die antikomplementäre Wirkung der Lösung aufgehoben.

6) Die inaktivierende Substanz des Cobragifts wird aus der wässerigen Lösung durch Sättigung mit Kochsalz gefällt.

7) Die inaktivierende Wirkung des Cobragifts wird durch konzentriertes Kochsalz gehemmt.

Nachdruck verboten.

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Studies on the Blood of two Cases of Paroxysmal Hemoglobinuria.

I. The Mechanism of the Autohemolytic Process.

By **R. A. Cooke.**

(Eingegangen bei der Redaktion am 25. Februar 1914.)

In 1904 Donath and Landsteiner[1]) demonstrated, in the blood of patients exhibiting the clinical symptoms of paroxysmal hemoglobinuria, a substance that was capable, at low temperatures, of sensitizing the patient's own red blood corpuscles to the destructive influence of human complement. In an article by J. Eason[2]) published in 1906, is an implied claim to priority of publication upon this property of hemoglobinurics blood, and this claim has had considerable public recognition (R. A. Cooke, loc. cit. below and particularly C. H. Browning and H. F. Watson, Journ. of Pathology and Bacteriology, Vol. 17, 1912). That this assumption of priority was unjustified has recently been demonstrated by Donath and Landsteiner[3]) and acknowledged by Eason himself.

The investigators that subsequently studied the blood in this disease generally confirmed the observations of Donath and Landsteiner, but arrived at conflicting conclusions regarding certain details in the mode of action of the autohemolysin.

Since none of the previous experiments has been carried out with the use of the usual quantitative methods of studies upon hemolytic processes, it seemed possible to us that the employment of those methods would enable us to explain the

1) Münch. med. Wochenschr., 1904, No. 36.
2) Edinburgh med. Journ., Vol. 19, p. 43.
3) Zeitschr. f. Immunitätsf., Bd. 18, p. 701.

apparent contradictions and finally to determine the manner of action of the autohemolysin. With this purpose, therefore, we have studied the blood of a case of paroxysmal hemoglobinuria that was under our observation during the winter of 1912—1913. The clinical aspect of the case has been treated in a separate publication [1]). The present paper is concerned especially with the mechanism of the autohemolytic phenomenon, which characterizes the disease.

The patient, who supplied the material for the investigation is a negro 38 years of age; he is single and denies all venereal history. In November 1902 — two years after coming to New York city from the West Indies, where he was born — he experienced a severe chill and fever, which was soon followed by the passing of black urine. Twelve hours afterward he was as well as usual and the appearance of the urine was normal. After an interval of one week a second attack occurred and since that time the attacks have recurred at greater or smaller intervals, being always accompanied with severe lancinating pain in the lumbar region, especially on the right side, and with a sensation of tingling and numbness in the extremities. The patient himself noticed that attacks followed exposure to draughts or immersion of the hands in cold water.

The Wassermann test resulted positively. Two injections of salvarsan (0.5 gr. and 0.4 gr. respectively) failed to prevent subsequent attacks or to change the result of the Wassermann tests. The "luetin" test, carried out by Dr. Noguchi one week after the second injection of salvarsan resulted positively.

For convenience of description we have employed in our report the following abbreviations:

"P" = the patient under observation.
"P's Ser." = the serum of the patient obtained by centrifugating the defibrinated blood while it was still warm.
"P's Ex. I Ser." = the serum of the patient obtained by the rapid centrifugation of the defibrinated blood, which had been subjected to a temperature of less than 4° C for one hour.
"P's Ina. Ser." = "P. Ser." heated for 20 minutes at 55° C.
"N. H. S." = normal human serum obtained from defibrinated blood.
"N. O°. S." = normal human serum obtained from blood that had been treated exactly as for "P. Ex. I Ser."
"G. P. Ser." = serum obtained from fresh defibrinated guinea-pig's blood.
"5 % P's Bl. C." = a 5 % suspension in physiological salt solution of the patient's washed blood corpuscles (based on the volume of corpuscular sediment).
"5 % N. H. C." = a similar suspension of normal human blood corpuscles.

1) American Journ. of the Med. Sc., Vol. 144, 1912.

The Donath-Landsteiner Experiment.

We have remarked that the demonstration of the autohemolysin in paroxismal hemoglobinuria by the method of Donath-Landsteiner has been generally successful in the hands of subsequent investigators. Their experiment, however, sometimes fails, and Meyer and Emmerich[1]) have shown that the failure is due, in some instances, to a lack of sufficient complement. The lack of complement was shown in two ways: first, by the failure of the serum to dissolve sensitized sheep's blood cells and secondly, by the hemolysis that resulted upon the addition of normal human serum to the hemoglobinuric's blood, followed by chilling and incubation at 37° C.

Occasionally, even the addition of normal human serum was ineffective in revealing the presence of the autohemolysin. The serum in one such case was found by Meyer and Emmerich to contain an unusual amount of anticomplementary substances and the presence of these substances in the serum was considered by them to be the possible cause of the failure of the Donath-Landsteiner experiment in that case.

This view has recently received experimental support from Kumagai and Inoue[2]). These writers describe cases of paroxismal hemoglobinuria in which the Donath-Landsteiner experiment failed even upon the addition of normal human serum. In every such instance, however, hemolysis could be obtained if, after chilling and before the addition of the non-hemolytic normal serum, the corpuscles of the hemoglobinuric's blood has been freed from the serum by washing with ice-cold salt solution.

Meyer and Emmerich describe a case in which even light attacks were accompanied with a complete exhaustion of complement; within 24 hours after the attacks, however, a considerable restoration of the complement content of the blood had already taken place. The authors sought to make use of these conditions in order to demonstrate the local production of complement. During the period of complement exhaustion, a finger was ligated and immersed for 10 minutes in ice-water.

1) Deutsches Arch. f. klin. Med., Bd. 96, 1909, p. 28.
2) Deutsche med. Wochenschr., 1912, No. 8.

At the end of this time the blood from the chilled finger was found to contain demonstrable complement whereas in the blood from another finger, which had not been ligated nor chilled, complement was not demonstrated.

If these results had been obtained by simple ligation of a finger without chilling it they would carry greater weight, for we have found that by merely chilling the defibrinated blood of some normal human beings the complementary strength of the serum is greatly increased. In two instances (sera A and B) this increase amounted to exactly 100 %. In one further case (serum C) however, no difference in complementary strength could be obtained by chilling the blood [1]).

Table I.

Showing an increase of the complementary activity of some normal human sera that had been kept for 1 hr. at a temperature of less than 4° C in contact with human corpuscles.

Hemolysis of 0.25 c. c. of a 5 % suspension of sensitized sheep's corpuscles when combined with.

Amount of serum c. c.	N. H. S. A	N. O°. S. A	N. H. S. B	N. O°. S. B	N. H. S. C	N. O°. S. C
0.1	c.	c.	c.	c.	c.	c.
0.05	v. str.	c.	str.	c.	c.	c.
0.025	sl.	v. str.	sl.	str.	mod.	mod.
0.0125	tr.	sl.	0	sl.	tr.	tr.

c. complete hemolysis; v. str. very strong hemolysis; mod. moderate hemolysis; sl. slight hemolysis; tr. trace of hemolysis.

In our own case we were always able, with the use of the unmodified method of Donath and Landsteiner, to demonstrate the autohemolysin.

The interaction of human corpuscle and autohemolysin.

The absorption of the autohemolysin at low temperatures by the individual's own blood corpuscles was first demon-

1) Donath and Landsteiner*) state that they were unable to observe an increase in the complement content of the blood as a result of chilling it. Our own failure in the one case may explain their lack of success.

*) Münch. med. Wochenschr., 1904, No. 36, p. 1592, food-note.

strated by Donath and Landsteiner [1] through the following experiment:

Plasma "A" obtained by centrifugation from blood "A" of the hemoglobinuric patient that had been chilled for one-half hour, was mixed with the corpuscular sediment of blood "B" (obtained from the same source) that had not been chilled and this mixture was incubated at 37 C., in one instance after first being chilled for one-half hour, in another instance without previous chilling. In both instances the corpuscles remained completely intact. On the other hand, when a mixture of plasma "B" with corpuscular sediment "A" was incubated without previous chilling, hemolysis occurred.

Donath and Landsteiner concluded from this experiment that the autohemolysin is absorbed by the blood corpuscles, which can then be dissolved by the addition of any human complement. These investigators [2]) were able, also, to reactivate the inactivated autohemolytic serum (20 minutes at 50° C.) by the addition of normal human serum.

All of those who subsequently studied the absorption of the autohemolysin by the corpuscles agreed with Donath and Landsteiner that this does take place at relatively low temperatures; but Moss [3]) in a recent contribution expresses the belief that in addition to low temperature the presence of complement is necessary for the absorption. This view is based by Moss upon the following experiments:

I. When corpuscles and inactivated autohemolytic serum are combined in a certain proportion and chilled for one-half hour, if the mixture be then warmed for five minutes the further incubation at 37° C. for two hours after the addition of normal human serum does not result in hemolysis; if, after the two hours incubation, the mixture be again chilled and again incubated, hemolysis then takes place.

II. If, in the experiment just described, after the first chilling and before complement is added, the corpuscles are freed from the serum by washing with cold salt solution, the corpuscular sediment cannot then be dissolved by incubating with normal human serum even after a second chilling.

III. If corpuscles and active autohemolytic serum be combined (in proportion very different from that of the preceding experiments $^1/_4$ to $^1/_{10}$ the relative amount of serum) and the steps of experiment II be otherwise followed, the cold-washed sediment does not dissolve if incubated without further treatment, but hemolysis does result from the incubation with added human complement even without a second chilling.

1) loc. cit.
2) Zeitschr. f. klin. Med., Bd. 58, 1906.
3) Johns Hopkins Hosp. Bull., Vol. 22, Juli 1911, No. 245.

In the fluid decanted from the corpuscle-active autohemolytic serum mixture after the first chilling, complementary function could be demonstrated.

On account of the absence from the protocols of any determination of hemolytic strength (either with respect to sensitizing or to complementary substances) of the various fluids employed in the three experiments, we cannot analyse the results. It is possible, however, without that information to show that the assumption of Moss (that the autohemolysin is absorbed from the serum by the corpuscles only in the presence of active complement) is superfluous.

We have been able, in fact, to demonstrate the absorption, by the corpuscles, of the autohemolysin from the hemoglobinuric's inactivated serum.

This was accomplished by a simple determination of the autohemolysin content of the hemoglobinuric's inactivated serum before and after its exposure for one-half hour at 0° C. to the washed corpuscular sediment of the patient's blood. The results of this comparative test is shown in Table II.

Table II.

Absorption of the autohemolysin from P's inactivated serum by P's blood corpuscles.

Amount of serum	In each tube 0.3 c. c. of N.H.S. + 0.25 c. c. 10 % P's corpuscles.	
	P's ina. ser.	P's serum inactivated after contact at 0° C. for ½ hr. with P's corpuscles [1])
ccm	Hemolysis after ½ hr. at 0° C. and ½ hr. at 37° C.	
0,4	complete [2])	moderate
0,2	„	slight
0,1	nearly complete	0
0,05	very strong	0
0,025	slight	—
0,0125	0	—

1) 1.2 c. c. (6 minimal complete hemolytic amounts) of P's inactive serum had been kept at 0° C. for ½ hr. in contact with the corpuscular sediment of 6.0 c. c. (4 times 6 units) of a 5 % suspension of P's blood corpuscles and the serum was then recovered by rapid centrifugation at low temperature.

2) Without the addition of normal human serum but under otherwise identical conditions this quantity of P's inactive serum caused no hemolysis.

It is seen that whereas 0.05 c. c. of P's inactive serum contained an amount of autohemolysin sufficient, under the conditions of the experiment, to cause very strong hemolysis, the same serum, after contact at low temperature with P's corpuscles, was able to cause only moderate hemolysis with 8 times that amount (0.4 c. c.). It seems, therefore, that about 85 % of the autohemolysin was removed from the complement-free P's serum during the contact with the human corpuscles.

No attempt has been made by us to explain Moss's results; Meyer and Emmerich believe they were due to a dissociation of the corpuscle-autohemolysin combination at some stage of his procedure.

Dissociation of the corpuscle-autohemolysin combination.

The corpuscle-autohemolysin combination is a relatively unstable one. This was first recognized by Moro[1], who found that the combination is dissociated at 37° C. Moro's observation was confirmed by Meyer and Emmerich and we, also, have been able to demonstrate a dissociation of the combination at 37° C. even when the amount of the autohemolysin that had been absorbed by the corpuscles was only one-tenth of a complete hemolytic unit.

1.0 c. c. (i. e., 2½ complete lytic amounts) of P's inactive serum was mixed with the sediment of 6¼ c. c. (i. e., ten times 2½ units) of a 5 % suspension of P's blood corpuscles; the mixture, after being chilled for ½ hr., was centrifugated, while still cold and the decanted serum "A" was compared, as to its autohemolysin content, with the decanted serum "B" from a similar mixture that had been incubated for ½ hr. at 37° C after having been chilled.

The results of the titration are shown in Table III (see p. 630).

Under the conditions of the preceding experiment about 90 % of the autohemolysin was absorbed by the corpuscles at the lower temperature and about 25 % was dissociated during the subsequent incubation at 37° C.

1) Münch. med. Wochenschr., 1909, No. 11.

Table III.

Demonstration of the dissociation of the corpuscle-autohemolysin combination.

	In each tube 0.3 c. c. "N. H. S." and 0.25 c. c. "5 % P's Bl. C." (First at: 0° C. ½ hour, then at 37° C)		
c. c.	Decanted "P's ina. ser." A.	Decanted "P's ina. ser." B.	"P's ina. ser." "P's ina. ser."
0.4	trace	partial hemol.	complete hemol.
0.1	0	slight „	very strong hemol. [1])
0.1	0	trace „	strong hemol.
0.05	0	trace „	slight „
0.0	—	—	0

The behaviour of complement.

Influenced, perhaps, by the well-known behaviour of complement and sensitizing substance in normal as well as immune hemolytic sera (the sensitizing substance having the property of combining with the corpuscles at low temperatures, whereas complement remains free under this condition) Donath and Landsteiner thought that in hemoglobinurics' sera, also, the corresponding substances that are responsible for the autohemolytic process are subject to the same law. They believed that here the autohemolysin united with the corpuscles only at low temperatures but that complement entered the sensitized corpuscles at the usual higher temperatures. Later, this question was investigated experimentally by Meyer and Emmerich, who found that both the autohemolysin and complement are taken up by the human corpuscles at low temperatures, that is, they showed that after being kept at a low temperature in contact with human corpuscles the hemoglobinuric's serum was no longer able to dissolve sensitized sheep's corpuscles. Moss's contrary conclusion was based on experiments that do not permit a quantitative analysis.

The results of our own study of the relations of complement in the homoglobinuric's serum confirm the obser-

1) The autohemolytic activity of P's serum varied somewhat from day to day (compare Table II).

vations of Meyer and Emmerich. In the case at our disposal P's serum dissolved 0.25 c. c. of 5% sheep's corpuscles completely in a quantity of 0.05 c. c. and moderately in a quantity of 0.025 c. c., whereas 0.4 c. c. of "P's Ex. I serum" caused no hemolysis at all with the same amount of the sensitized sheep's corpuscles. From the result of this experiment it may be estimated that 95%, at least, of the respective complement had been removed during the exposure of P's defibrinated blood to cold. Combined with 0.25 c. c. of 5% P's blood corpuscles, 0.4 c. c. of "P's Ex. I serum" produced no hemolysis. That this loss of hemolytic power was due to a lack of complement is made probable by the fact that with the addition of 0.3 c. c. of fresh normal human serum, 0.2 c. c. of P's Ex. I serum produced strong hemolysis of 0.25 c. c. of 5% P's blood corpuscles. It is evident, therefore, that during the exposure of P's defibrinated blood to a low temperature the autohemolysin and also complement capable of dissolving the sensitized human corpuscles are removed from the serum.

Zusammenfassung.

1) Durch Abkühlung des defibrinierten Blutes von manchen normalen Menschen wird der Komplementgehalt des Serums um 100 Proz. erhöht.

2) Das Autohämolysin der Hämoglobinuriker verbindet sich mit den menschlichen Blutkörperchen nur bei niederen Temperaturen (Donath und Landsteiner u. a.) und diese Verbindung wird bei höheren Temperaturen wieder teilweise gelöst (Moro u. a.).

3) Das Komplement, daß an dem autohämolytischen Vorgang teilnimmt, wie auch dasjenige Komplement, das sensibilisierte Hammelblutkörperchen auflöst (Meyer und Emmerich) wird durch die Autohämolysin-Blutkörperchenverbindung bei niederen Temperaturen absorbiert.

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Studies on the Blood of two Cases of Paroxysmal Hemoglobinuria.

II. The Multiplicity of Complement.

By A. F. Coca and R. A. Cooke.

(Eingegangen bei der Redaktion am 25. Februar 1914.)

The question whether, in the same serum, the complementary function is exercised by only one group of substance or by a number of such substances has been the subject of considerable study and, also, of dispute. Most of those who have approached the question experimentally have accepted the view, first advanced by Ehrlich and Morgenroth, that there may be in the same serum more than one complementary substance or system. However, Bordet[1], in his latest publication upon the subject, still maintains his belief in the unity of complement.

All the experimental studies and theoretical discussions of the question have been thoroughly and critically reviewed by Sachs[2]), and as no new facts have been added to the subject since that review was published it does not seem necessary to discuss the previous work here.

Most of the studies bearing upon the question of the plurality of complement were conducted previous to the demonstration of the complex constitution of complement. Therefore, assuming the plurality of complement, the further question now suggests itself whether the different complementary

1) Zeitschr. f. Immunitätsf., Ref., Bd. 1. 1909. p. 29.
2) Kraus-Levaditi Handb., Bd. 2, p. 922.

systems in the same serum are distinct in all of their demonstrated components or only in certain of them.

For the investigation of this question a particularly favorable material is found in the blood of those affected with paroxysmal hemoglobinuria. It has been observed, namely, by Meyer and Emmerich that after mere exposure to a low temperature, the defibrinated blood of such an individual loses completely its hemolytic or complementary activity with respect to sensitized sheep's corpuscles, while still retaining its natural hemolytic activity with respect to rabbit's corpuscles.

These conditions are especially favorable inasmuch as the process of separation of the two complementary systems is the simplest conceivable; furthermore, the separation is apparently quantitative; and, finally, the complementary function that is extinguished is more powerful than the one that remains, a circumstance that satisfies one of Bordet's strongest objections to the acceptance of a multiplicity of complement.

For a short period during the winter ('13—'14) we had the opportunity of examining the blood of a second case of paroxysmal hemoglobinuria, and with that material we were able satisfactorily to analyse the two complementary functions referred to above.

In describing the experiments involved in this investigation we have used the same abbreviations that were employed in the preceding study (p. 2, I).

As a preliminary experiment, "P's Ex. I serum" was compared with P's serum as to its hemolytic activity when combined, on the one hand with sensitized sheep's corpuscles, and on the other hand with rabbit's corpuscles. The results of these tests, which are shown in table I confirm the observation of Meyer and Emmerich to which we have referred. Whereas at least 97 % of the complementary activity of P's serum against sensitized sheep's corpuscles was lost by the exposure of the blood to cold, the natural hemolytic activity of the blood against rabbit's corpuscles was retained in considerable part — about 50 %.

Table I.

Showing the effect on the hemolytic activity of the hemoglobinuric serum caused by chilling the defibrinated blood for 1 hr. at 0° C.

Amount of serum	In each tube 0.1 c. c. of a $2^1/_2$ % suspension of sensitized sheep's corpuscles		In each tube 0.1 c. c. of a $2^1/_2$ % suspension of rabbit's corpuscles	
	Degree of hemolysis produced by			
c. c.	P's serum	P's Ex. I serum	P's serum	P's Ex. I serum
$^4/_{10}$	—	0	—	—
$^2/_{10}$	c.	0	c.	c.
$^1/_{10}$	c.	—	c.	c.
$^1/_{20}$	c.	—	c.	n. c.
$^1/_{40}$	c.	—	n. c.	0
$^1/_{80}$	n. c.	—	sl.	0
$^1/_{160}$	0	—	0	—

c. = complete hemolysis, n. c. = nearly complete hemolysis, sl. = slight hemolysis, 0 = no hemolysis.

That this reduction in the anti-rabbit hemolytic activity was not due to a diminution in the amount of the normal anti-rabbit amboceptor was demonstrated by determining the relative amount of that substance in P's serum and "P's Ex. I serum" with the addition of an excess of rabbit's complement.

The result of the examination is shown in table II.

Table II.

Showing that exposure of P's defibrinated blood to low temperatures does not diminish the amount of natural anti-rabbit corpuscle amboceptor.

Amount of serum	In each tube 0.2 c. c. of normal rabbit's serum and 0.1 c. c. of a $2^1/_2$ % suspension of rabbit's corpuscles	
	Degree of hemolysis produced by	
c. c.	P's serum	P's Ex. I serum
$^1/_{10}$	complete	complete
$^1/_{20}$	"	"
$^1/_{40}$	"	"
$^1/_{80}$	nearly complete	nearly complete
$^1/_{160}$	moderate	moderate
$^1/_{320}$	slight	slight

By this method of examination the amount of natural anti-rabbit amboceptor was found to be the same in P's serum and "P's Ex. I serum". The reduction in the anti-rabbit hemolytic

activity of the hemoglobinuric's serum that resulted from chilling the defibrinated blood must have been due, therefore, to some influence upon the complementary substances.

There was sufficient indication in the results of the two preceding experiments that the anti-rabbit hemolysin of "P's Ex. I serum" is a complex one consisting of complementary substances and amboceptor or sensitizing substance. This fact was more thoroughly established, however, by means of the cold-separation method in the following experiment.

4.0 c. c. of "P's Ex. I serum" and the washed sediment of 2.0 c. c. of rabbit's blood were chilled to 0° C and then mixed. The mixture was kept at 0° C for one hour, during which time no solution of the corpuscles took place. The serum that was then recovered by rapid centrifugation was designated "P's Ex. II serum", and it was compared with "P's Ex. I serum" as to its amboceptor and complement content with respect to rabbit's corpuscles.

The results of these tests are shown in table III.

Table III.

Demonstrating the complex nature of the natural anti-rabbit's corpuscle hemolysin of human serum.

Amount of the different Preparations of P's Serum	In each tube 0.25 c. c. of a 5 % suspension of rabbit's blood corpuscles								
	In each tube 0.25 c. c. of rabbit's serum						In each tube 0.4 c. c. inact. P's Ex. I Ser.		
	P's Ser.	P's Ex. I Ser.	P's Ser.	P's Ex. I Ser.	Inactivated P's Ex. I S.	P's Ex. II Ser.	P's Ex. II Ser.	P's Ex. I Ser. and	inactive P's Ex. I Ser.
	1.	2.	3.	4.	5.	6.	7.	8.	
c. c.	Degree of hemolysis after 2 hours at 37° C								c. c.
0.4	complete	complete	complete	complete	complete	complete	complete	complete	0.0
0.2	„	strong	„	„	„	moderate	„	„	0.2
0.1	strong	slight	„	„	„	trace	slight	moderate	0.3
0.05	slight	0	„	„	strong	0	trace	slight	0.35
0.025	0	0	moderate	slight[1])	trace	0	0	0	0.4
0.8					Rabbit's ser. omitted 0	0			

1) When these tests — 3 and 4 — were made with the use of relatively less — 0.1 c. c. — of the suspension of rabbit's corpuscles this small apparent difference in the natural anti-rabbit blood amboceptor of the two serum preparations disappeared, the results being identical for both as follows 0.025 = complete, 0.0125 = nearly complete, 0.00625 = moderate, 0.003125 = slight. Compare with table II.

Analysis of table III.

The results shown in columns 1 and 2 confirm those in the third and fourth columns of table I; that is, they demonstrate that by the chilling of the hemoglobinuric's defibrinated blood the general hemolytic activity of the serum with respect to rabbit's corpuscles was diminished by one-half.

Comparing columns 4 and 6 we see that at least 75 % of the anti-rabbit amboceptor was removed from "P's Ex. I serum" by exposure at low temperature to the washed corpuscular sediment of rabbit's blood.

Heating of "P's Ex. I serum" for one-half hour at 52° C weakened the sensitizing power of the serum with respect to rabbit's corpuscles (column 5).

Columns 3 and 4 respresent a repetition of the comparative test shown in table II, the results of which are here confirmed.

The purpose of the tests recorded in columns 7 and 8 was to compare "P's Ex. I serum" and "P's Ex. II serum" as to their complementary activity against sensitized rabbit's corpuscles. Since "P's Ex. II serum" contained relatively little anti-rabbit amboceptor, this function was supplied (in column 7) by the addition, to each tube, of 0.4 c. c. of inactivated "P's Ex. I serum". In testing the complement content (anti-rabbit) of "P's Ex. I serum", since "P's Ex. I serum contained the anti-rabbit amboceptor in full concentration, that function was added proportionately as the quantity of "P's Ex. I serum" (column 8) diminished. In this way the amount of anti-rabbit amboceptor in each tube of series 7 and 8 was made practically constant. The results of this comparative test seem to show that "P's Ex. II serum" contained a little less of the complement in question than did "P's Ex. I serum". Some of this difference, however, may have been due to the use, in the 3d and 4th tube of series 7, of somewhat more of the inactivated "P's Ex. I serum" than in the corresponding tubes of the 8th series, and it is probable that the inactivated serum exercised an anticomplementary effect.

However this may be, the whole experiment demonstrates plainly the complex nature of the natural anti-rabbit corpuscle hemolysin of "P's Ex. I serum", over 75 % of the sensitizing substance being removed at 0° C by rabbit's corpuscles, whereas the corresponding complementary substances were little if at all influenced under the same circumstances.

Having thus demonstrated the complex nature of the anti-rabbit corpuscle hemolysin of the hemoglobinuric's serum we procceded to the analysis of the two complementary functions of that serum to which we have referred.

For the purpose of conducting this proposed investigation other serum preparations in addition to these already employed in the preceding experiments were necessary. These preparations, with their abbreviations, were:

1) Normal human serum end-piece solution = "N. H. endpiece".
2) „ „ „ mid-piece solution = "N. H. midpiece".
3) P's serum end-piece solution = "P's ser. endpiece".
4) „ „ mid-piece „ = "P's ser. midpiece".
5) P's Ex. I serum end-piece solution = "P's Ex. I ser. endpiece".
6) P's Ex. I serum mid-piece „ = "P's Ex. I ser. midpiece".

In every instance these solutions were prepared with the use of the carbon-dioxide method of Liefmann. The sera were always mixed with nine parts of distilled water, and carbon-dioxide gas was passed for five minutes through the diluted sera. The precipitate was then separated by means of rapid centrifugation for ten minutes from the soluble portion, which could then be obtained quantitatively by decantation. The clear decanted fluid, which contained the "end-piece" of complement, was made isotonic with the addition of a quantity of 9.0 % sodium chloride solution that was equal to one-tenth of the volume of distilled water that had been used in diluting the serum. This preparation, which was designated "end-piece solution", represented approximately a 9 % dilution of the original serum. The centrifuge tube, which still contained the precipitate as a compact mass adhering to the bottom of the tube, was rinsed two or three times with distilled water and the entire precipitate was then rubbed up with a glass rod and shaken a little with a quantity of physiological saline solution that was equal to twice the volume of the original serum used. This preparation, which was designated "mid-piece solution", represented volumetrically a 50 % solution of the mid-piece that was contained in the original serum.

7) P's "yeast-serum" = "P's yeast ser.".

This preparation represented the serum of the hemoglobinuric patient that had been deprived of all of the third component of complement with the use of yeast[1]). One volume of a 20 % suspension of washed yeast cells (24 to 48 hour saccharose-wort-agar culture) was mixed with two volumes of serum and the mixture was placed in the incubator at 37° C for one hour. The yeast cells were kept in uniform suspension by gentle shaking of the mixture every ten minutes. By centrifugation the yeast cells were easily separated from the fluid, which was decanted and designated "P's yeast ser."

8) Inactivated sera = "ina. ser."

The different sera were rendered inactive by being heated for $^1/_2$ hour at 52° C.

Two suspensions of washed blood corpuscles were employed as indicators in the following experiments. One suspension was of rabbit's corpuscles and the other was of sheep's corpuscles with which had been mixed four or five complementary hemolytic doses of antisheep's corpuscle amboceptor obtained from an immunized rabbit. Both suspensions were of $2^1/_2$ % dilution estimated upon the volume of corpuscular sediment. In all of the tests the same volume of these corpuscle suspensions was used for each tube: that is $^1/_{10}$ c. c.

1) Zeitschr. f. Immunitätsf., Bd. 21, 1914, p. 604.

In table IV (a and b) are shown the results of a quantitative comparison of P's serum and "P's Ex. I serum" with respect to the three components of the complementary system that is directed against sensitized sheep's blood corpuscles.

Table IVa.

A quantitative comparison of P's serum and P's Ex. I serum with respect to the three components of the complementary system that is directed against sensitized sheep's corpuscles.

In each tube		Hemolysis of $^1/_{10}$ c. c. of a 2% suspension of sensitized sheep's blood corpuscles after 2 hrs. at 37° C.							
		$^1/_{10}$ c. c.	$^1/_{20}$ c. c.	$^1/_{40}$ c. c.	$^1/_{80}$ c. c.	$^1/_{160}$ c. c.	$^1/_{320}$ c. c.	$^1/_{640}$ c. c.	$^1/_{1280}$ c. c.
1) $^1/_{10}$ c. c. N. H. midpiece (A = N. H endpiece)	+ A	—	c.	c.	c.	c.	—	—	—
2) $^1/_{20}$ c. c. N. H. endpiece (B = N. H. midpiece)	+ B	—	c.	c.	c.	st.	—	—	—
3) $^1/_{10}$ c. c. P's Ex. I Ser. A = N. H. endpiece B = N. H. midpiece	+ A	c.	c.	st.	m	—	—	—	—
	+ B	0	0	0	0	—	—	—	—
4) $^2/_{10}$ c. c. N. H. endpiece B^1 = P's Ser. midpiece B^2 = P's Ex. I Ser. midpiece	+ B^1	c.	c.	c.	c.	c.	m	sl.	—
	+ B^2	c.	c.	v. st.	sl.	0	0	—	—
5) $^1/_{10}$ c. c. P's yeast Ser. D = P's Ex. I Ser. E = P's ina. Ser.	+ D	—	—	—	c.	c.	c.	—	st.
	+ E	—	—	—	c.	c.	c.	—	—

Table IVb. Control tests.

	Hemolysis of $^1/_{10}$ c. c. of a 2% suspension of sensitized sheep's corpuscles, after 2 hrs. at 37° C.					
	$^1/_{10}$ c. c.	$^1/_{20}$ c. c.	$^1/_{40}$ c. c.	$^1/_{80}$ c. c.	$^1/_{160}$ c. c.	$^1/_{320}$ c. c.
1) P's Serum	—	—	c.	c.	c.	st.
2) P's Ex. I Serum	0	0	0	—	—	—
3) P's ina. Serum	0	0	0	—	—	—
4) P's yeast Serum	0	0	—	—	—	—
5) N. H. endpiece	sl.	0	0	0	—	—
6) N. H. midpiece	0	0	0	—	—	—

Tests No. 1 and 2 show that the preparations of normal human serum end-piece and mid-piece contained those components of the anti-sheep's corpuscle complement in quantity sufficient for the requirements of the subsequent tests.

Test No. 3 shows that the anti-sheep's corpuscle end-piece is entirely lacking in "P's Ex. I serum"

· $^1/_{10}$ c. c. of P's serum contained about 6 completely hemolytic doses of anti-sheep's corpuscle complement (see table IV b first row), yet in the same quantity of "P's Ex. I serum" there was not enough end-piece to produce the least hemolysis of the unit of sensitized sheep's corpuscles when combined with 8 completely hemolytic amounts of normal human mid-piece. (Table IVa, test 3, row B.)

The same test shows (row A) that the corresponding mid-piece is present in "P's Ex. I serum"; but the comparative test No. 4, table IVa, demonstrates that between 80 and 85 % of this component of the anti-sheep's corpuscle complement was removed from P's serum during its contact, at low temperature, with the human corpuscles.

The presence of a considerable quantity of the third component of the anti-sheep's corpuscle complement in "P's Ex. I serum" is shown in table IVa, test No. 5. This test seems also to indicate that P's serum contained one-half as much of the third component as did "P's Ex. I serum", but the apparent paradox is due to the fact that in the test P's serum was used in the heated conditions whereas "P's Ex. I serum" had not been heated. We have been unable to repeat this comparative test with serum preparations that have had identical previous treatment (that is, both heated or both unheated) but it is permissible, perhaps, to estimate the relative amount of the third component (anti-sheep's corpuscle) with the aid of the results of test No. 8 in table Va. This test shows that the heating of P's serum and of "P's Ex. I serum" diminishes the activity of the third component that is directed against rabbit's corpuscles to $^1/_4$ of its original strength and if that component of the complementary system that is directed against sheep's corpuscles is similarly affected by heating, the apparent relation of the amounts of the third component as determined in test No. 5, table IVa, would be exactly reversed; that is, there was just one-half as much of the third component (anti-sheep) in "P's Ex. I serum" as there was in P's serum.

In the preceding quantitative study of the three components of the complementary system that is directed against sensitized sheep's corpuscles it was found that during the exposure of P's defibrinated blood to cold,

1) All of the end-piece was removed.
2) The mid-piece activity was reduced to 15 or 20% of its original strength.
3) The third component activity was probably diminished by 50%.

In table V (a and b) are the results of a quantitative comparison of P's serum and "P's Ex. I serum" with regard to the three components of the complementary system that is directed against rabbit's blood corpuscles.

Table Va.

A quantitative comparison of P's serum and P's Ex. I serum with respect to the three components of the complementary system that is directed against rabbit's corpuscles.

In each tube		Hemolysis of $^1/_{10}$ c. c. of a $2^1/_2$% suspension of rabbit's blood corpuscles, after 2 hrs. at 37° C.							
		$^1/_{10}$ c. c.	$^1/_{20}$ c. c.	$^1/_{40}$ c. c.	$^1/_{60}$ c. c.	$^1/_{80}$ c. c.	$^1/_{100}$ c. c.	$^1/_{120}$ c. c.	$^1/_{140}$ c. c.
6) $^2/_{10}$ c. c. P's Ser. midpiece A = P's Ser. endpiece A¹ = P's Ex. I Ser. endpiece	+ A	c.	m	0	0	—	—	—	—
	+ A¹	c.	m	0	0	—	—	—	—
7) $^4/_{10}$ c. c. P's Ser. endpiece B = P's Ser. midpiece B¹ = P's Ex. I Ser. midpiece	+ B	c.	c.	c.	c.	c.	c.	st.	sl.
	+ B¹	c.	c.	c.	c.	sl.	0	0	0
8) $^1/_{10}$ c. c. P's yeast Ser. D = P's Ser. (not heated) D¹ = P's Ex. I Ser. (not heat.) E = P's ina. Ser. E¹ = P's ina. Ex. I Ser.	+ D	—	—	c.	c.	c.	c.	sl.	0
	+ D¹	—	—	c.	c.	c.	sl.	0	0
	+ E	—	—	c.	c.	0	0	0	—
	+ E¹	—	—	c.	c.	0	0	0	—

Table Vb. Control tests.

	Hemolyse of $^1/_{10}$ c. c. of a $2^1/_2$% suspension of rabbit's blood corpuscles, after 2 hrs. at 37° C.					
	$^1/_{10}$ c.c.	$^1/_{20}$ c.c.	$^1/_{40}$ c.c.	$^1/_{60}$ c.c.	$^1/_{80}$ c.c.	$^1/_{100}$ c.c.
P's Serum	—	—	c.	c.	st.	tr.
P's Ex. I Serum			c.	st.	0	0
P's Ser. endpiece*)	0	0	—	—	—	—
P's Ser. midpiece	0	0	—	—	—	—
P's ina. Ser.	0	0	—	—	—	—
P's ina. Ex. I Ser.	0	0	—	—	—	—
P's yeast Ser.	0	0	—	—	—	—
P's Ex. I Ser. endpiece*)	0	0	—	—	—	—
P's Ex. I Ser. midpiece	0	0	—	—	—	—

*) $^1/_{10}$ c. c. of these preparation was also entirely non-hemolytic.

In test No. 6, table Va, the results of the titration of the anti-rabbit corpuscle end-piece in P's serum and in "P's Ex. I serum" show no difference in respect to that reagent in these two preparations of the hemoglobinuric's serum.

Test No. 7, table Va, on the other hand, shows that "P's Ex. I serum" contains between 80 and 85 % less of the anti-rabbit corpuscle mid-piece than does P's serum.

The comparison, in test No. 8, table Va, of the inactivated serum preparations as to their "third-piece" content shows that only one-half as much of this component was present in "P's Ex. I serum" as was found in P's serum. Test No. 8 shows, also, that the third-piece activity of the heated serum preparations is only one-fourth as great as that of the unheated sera.

This result agrees with the observation of Husler[1]) and Jonas[2]) both of whom found that the third-piece function is weakened by heat. Jonas found, furthermore, that human third component of complement is usually rendered completely inactive by being heated for 15 minutes at 55° C., whereas the corresponding function in pig's serum is only weakened by 50 % by exposure to 55° C. for $^1/_2$ hr. As has already been stated, all of the heated human sera used in our reactivation tests had been exposed for $^1/_2$ hr. to a temperature of 52° C.

A comparison of the results of the two foregoing series of tests shows that, as a result of the exposure of the hemoglobinuric's defibrinated blood to a low temperature,

1) all of the anti-sheep's corpuscle end-piece was removed from the serum, whereas the anti-rabbit's corpuscle end-piece remained apparently unchanged;
2) both the anti-sheep's corpuscle mid-piece and the anti-rabbit's corpuscle mid-piece suffered a reduction of between 80 and 85 %;
3) the anti-rabbit's corpuscle third component was diminished by 50 %, and the third component of the anti-sheep system of complement was probably reduced, also by 50 %.

1) Zeitschr. f. Immunitätsf., Bd. 15, 1912.
2) Zeitschr. f. Immunitätsf., Bd. 17, 1913.

On the basis of this comparison it appears that the anti-sheep's corpuscle end-piece and the anti-rabbit's corpuscle end-piece are two distinct substances neither of which can perform the function of the other in respect to its hemolytic action; furthermore it seems probable that in the two complementary systems that we have studied in the human serum, the mid-piece function is performed by a single substance, the same thing being probably true, also, of the function of the third component of complement.

In table I, it has been shown that chilling of the defibrinated blood of the hemoglobinuric patient causes a reduction in the general activity of the complementary system that is directed against rabbit's corpuscles. The subsequent experiences make it seem probable that this reduction is due to the absorption by the human corpuscles of part of the anti-rabbit mid-piece and third component of complement.

Zusammenfassung.

Im menschlichen Serum sind zwei Komplementsysteme nachgewiesen worden (Meyer und Emmerich). Durch eines dieser Systeme wird Hämolyse der sensibilisierten Hammelblutkörperchen und auch der mit dem Autolysin der paroxysmalen Hamoglobinurie sensibilisierten menschlichen Blutkörperchen verursacht. Das andere Komplementsystem mit dem im menschlichen Serum natürlich vorkommenden Anti-kaninchenblut-Ambozeptor zusammen, bewirkt Hämolyse der Kaninchenblutkörperchen.

Die Analyse dieser zwei Komplementsysteme hat gezeigt:

1) daß jedes System sein eigenes Endstück besitzt; d. h. die 2 Endstücke können einander nicht ersetzen;

2) daß beide Systeme ein gemeinsames Mittelstück haben, und

3) daß die Funktion der dritten Komponente in beiden Systemen wahrscheinlich von einer einzigen Substanz ausgeübt wird.

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TREATMENT OF TRANSPLANTABLE RAT SARCOMA BY FULGURATION

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WITHIN the last five years de Keating-Hart[2] has published a number of papers in which he has advocated the use of fulguration as a complement to operation in the treatment of malignant growths. Following his lead, many surgeons, both abroad and in this country, have employed the method with varying degrees of success. By some it has been condemned as of very little or no value, while others believe it to be a decided advance in cancer treatment.

De Keating-Hart mentions that many of the failures have been due to the use of unsuitable apparatus and to its employment in cases in which the method is not applicable.

In order to form a basis for the use of the method in the treatment of human tumors and to get some explanations for the varying opinions in regard to it, the writers have carried out an extensive series of animal experiments, the results of which form the basis of this paper.

The apparatus employed in these experiments was purchased in Paris from Gaiffe, the maker who has supplied de Keating-Hart with his apparatus; and before it was shipped to this country it was carefully examined, tested, and approved by de Keating-Hart, and is similar in all respects to that employed by the originator of the method. For these reasons it appears that no exception may be taken to the results of our experiments which is founded upon the criticism that faulty or unsuitable apparatus was employed.

De Keating-Hart insists that the current must be of sufficient voltage to give a spark 8 cm. long. He is very insistent that the spark must be cold, so that the tissue changes which follow its application are not due to a cauterizing effect. The field of usefulness of this method of treatment is limited to localized growths without general metastasis. The tumor must be removed surgically as completely as possible, complete hæmostasis secured, and the whole field of the operation then subjected to the action of the spark. The purpose, obviously, is to kill off both the gross and microscopic fragments of malignant tissue which have not been surgically removed.

The manner of accomplishing such a result is not fully understood. De Keating-Hart maintains that it is not due to an actual killing of the tumor tissue by the spark, but to a change in the nutritional conditions of the region treated, so that the tumor cells are not able to grow. The bearing of our experiments on such a conclusion will appear later.

The plan of these experiments includes a study of the effect of the current on normal tissues — the physiological effects obtained when stimulation is made over what may be called critical areas, such as the course of the large nerve trunks and the heart. Likewise a study has been made of the effects upon transplantable sarcoma of rats. It is these latter studies which make up the major portion of these experiments.

1. *The effect of the current on normal tissue.* The chest of several guinea pigs was shaved, and the pigs were anæsthetized and exposed to the high-frequency spark directly over the cardiac area for varying lengths of time, from 30 seconds to two minutes. The length of the spark varied from 8–12 cm. The animals suffered no serious damage or shock from this experience, and recovered from the anæsthetic with very little disturbing effect. In these preliminary tests, it must be remembered, the current was applied to the intact skin of the chest wall. A further series of animals was fulgurated over the neck region along the course of the vagus, the skin being intact. No disturbing effects whatsoever were ob-

[2] La fulguration et ses resultats dans le traitement du cancer. 2me edition. Paris, 1909, A. Moloine, Editeur.

[1] From the Huntington Fund for Cancer Research, Loomis Laboratory, Cornell University Medical College, New York.

served. It seemed to us that the current was diffused by the other tissues to such a degree that the heart was not influenced to any appreciable extent.

The next experiments were made upon dogs under ether anæsthesia. The carotid artery, internal jugular vein, and vagus nerve were exposed without, however, dissecting them free from their common sheath. Sparks applied along the course of the vessels caused practically no disturbance to the heart. The vagus was next dissected free and supported on two glass rods away from the other tissues. An 8-cm. spark was applied directly to the nerve for a period of 30 seconds. There was an immediate change in the respiration. There were very rapid respiratory movements; the heart continued to beat but was quite irregular. The nerve was then returned to its sheath and the wound closed. The animal recovered from the experiment promptly and appeared to suffer no permanent ill effects. Another animal, on which a similar experiment was made on both vagi at the same time, suffered extreme prostration, recovered very slowly, and died the next day. It seems evident that to cause any serious damage to the vagus it is necessary to apply the current directly to the nerve trunk. If the nerve is protected by the great vessels or the nerve sheath, the current is diverted to such an extent that practically no damage is done.

2. *Nature of the local reaction following fulguration.* The high-frequency spark produced by this apparatus is called a cold spark, because it does not immediately cauterize the tissue. The question arises as to how the effect is produced, if there is no local destruction of tissue. De Keating-Hart[1] is inclined to explain the effect as being due to some subtle physiological change which is not accompanied by marked anatomical alteration:

"La plupart ont crû voir dans la fulguration un moyen de destruction directe du cancer. Moi-même, au début, j'avais attaché à la sidération de la cellule cancéreuse par l'étincelle une importance que j'ai, depuis, reconnue illusoire. Or, les recherches histologiques nous ont montré que la *destruction*, quand elle existait, était superficielle. D'où il semblait naturel de conclure que la vertu curatrice de l'étincelle était à peu près nulle et que le seul effect qu'on dût attendre d'elle était de hâter la cicatrisation des plaies cancéreuses, enserrant ainsi l'élément pathologique dans les mailles d'un tissu fibreux défensif.

"Or, rien de ces deux effets, *destructeur* et *sclérogène*, ne me paraît suffisant à expliquer ce que nous observons. Je dirai plus: ni l'action cicatrisante, ni le pouvoir destructeur ne me semblent contribuer à la cure du cancer, *la première n'existant pas, le second ne devant pas être recherché* dans la plupart des cas.

"Examinons d'abord le second: le chirurgien a enlevé avant l'étincelage tout ce qu'il était possible de séparer, sans danger excessif, de l'être vivant. Les organes qui restent, s'ils ont droit au respect du bistouri, ne doivent pas attendre moins de l'étincelle, et celle-ci n'aura que de rares occasions de parachever l'œuvre chirurgicale; sauf en quelques cas exceptionnels donc, il sera inutile de produire une escarrification électrique.

"Quant à *l'action cicatrisante*, des apparences trompeuses y ont fait croire; mais l'expérience m'a conduit peu à peu à des conclusions contraires. Certes, de larges pertes de substance ont été sous nos yeux, après fulguration, rapidement comblées; mais si nous examinons de près le mécanisme de ces fermetures, nous pouvons constater qu'elles sont dues non tant à une reformation cellulaire intense qu'à un énergique appel centripète des tissus mous environnants. Quand ceux-ci s'y prêtent, les téguments sont rapprochés bord à bord et la cicatrice se fait assez vite avec un aspect *linéaire* qui dès les premières observations lui a valu le nom très juste d'autoplastie naturelle. Mais si, en revanche, la peau trop tendue ne peut couvrir la plaie, celle-ci, loin de continuer à se fermer, demeure, souvent des mois entiers, sans tendance à la cicatrisation, véritable ulcération saine mais *torpide*.

"De même, si l'on traite des *ulcères variqueux* à l'aide d'étincelles de haute tension à *dose et à puissance fulgurante*, on supprime leur aspect malsain, mais *on ne hâte pas leur cicatrisation;* loin de là, bien souvent je l'ai vue notablement retardée.

"Ni *cicatrisante*, ni *destructive*, comment donc expliquer l'action curative de l'étincelle sur le cancer?

"Nous en sommes encore en ce point réduit aux hypothèses. Sans entrer dans le détail des recherches que j'ai entreprises à ce sujet, je dirai simplement ici la façon dont je conçois les effets de la fulguration sur les tissus.

"L'étincelle de *haute tension à dose fulgurante* produirait sur ceux-ci *des effets d'ordre surtout physiologique*, sans altération anatomique marquée. Nous savons déjà que la foudre, les courants industriels puissants peuvent déterminer des paralysies temporaries *sine materia*, des troubles sensitifs ou psychiques, où le microscope non plus que la recherche des réactions de dégénérescence ne révèlent quoi que ce soit d'anormal. De même, l'étincelle de haute-tension, projetée sur une peau saine, produit momentanément des vasoconstrictions intenses, et, frappant le muscle sterno-cléido-

CHART 1

The chart gives in the shaded areas the comparative size of the tumors in the two areas. Careful measurements were made of the tumors with fine calipers every ten days, the first measurement being made ten days after the tumors were planted. The tumors in the upper line are those in the control area between the shoulders, the lower line those in the experimental area which had, before planting, been subjected to fulguration. In this chart are plotted the results obtained from three different animals, which are representative of the results obtained by treating a large number of animals. In the first animal no growth was obtained in the experimental area, while in the control area it grew well, showing that the animal was not immune. This animal is typical of a large number treated in the same fashion. In the second animal the plant was made just outside the experimental area, and the result shows positive growth, indicating that the inhibiting effect is confined strictly to the area of reaction following the spark. The third animal is typical of a small number of animals which showed no growth in the experimental area but in which the growth in the control area, although positive, quickly regressed.

In such animals we are either dealing with animals whose natural immunity is high, or their immunity has been increased by absorption of the graft planted in the experimental area.

mastoïdien ou le tendon d'Achille, un torticollis ou un équinisme pouvant durer plusieurs semaines et davantage. Que ce soit par une action spasmodique secondaire sur les vaisseaux, or pour toute autre cause, la fulguration produit aussi, nous l'avons dit une sorte de stupéfaction locale[1] retardant la cicatrisation, c'est-à-dire la pullulation de la cellule épithéliale saine au point frappé. N'est-il pas admissible que cette même action s'exerce avec une force plus grande encore, sur la pallulation néoplasique? Cela expliquerait les effets d'arrêt évolutif constatés souvent ainsi, non seulement sue la plaie opératoire mais autour d'elle, en des régions plus ou moins infiltrées de cancer où le bistouri n'est pas intervenu, mais assez superficielles pour avoir perçu le choc de l'étincelle promenée autour de la perte de substance."

In order to determine to what degree normal tissues are affected we fulgurated a number of guinea pigs on the abdominal wall over an area as large as a silver half-dollar for a period of 30 seconds to one minute, having a spark always at least 8 cm. in length. These pigs were killed at intervals of from one day up to six days after fulguration. The experimental area of the abdominal wall was removed and fixed in Müller's fluid for histological sectioning.

[1] Sidération sous-cancéreuse modifiant la nutrition du tissu et secondairement celle du cancer.

HISTOLOGICAL CHANGES

Twenty-four hours. There is extensive œdema of derma and subcutis, intense congestion of vessels, small hæmorrhages, and considerable exudation of polynuclear leucocytes. The subcutaneous muscle fibers are extensively vacuolated. The epidermis shows desquamation of cells.

Forty-eight hours. There is still much œdema and exudate. In the muscular layer there is beginning proliferation of endothelial cells of capillaries and of nuclei of the sarcolemma cells. In the epidermis there are small collections of leucocytes.

Seventy-two hours. The œdema of the derma and subdermal tissues is moderate. There is intense congestion of vessels and some extravasated blood. There are a few foci of polynuclear leucocytes.

Four days. The skin and deeper tissues are much thickened. The epidermis is extensively eroded and the surface of the derma is extensively infiltrated with leucocytes and blood. The loose subdermal tissues show active new growth of spindle cells. The outer layers of muscle tissue show a very

We are indebted to Dr. [illegible] of Cornell for the [illegible]

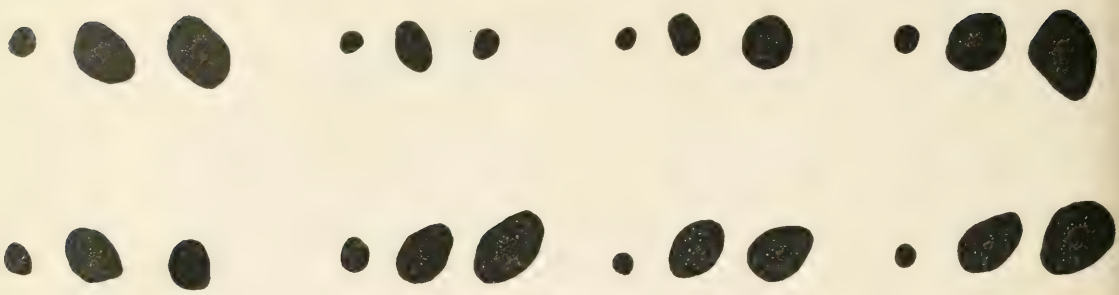

CHART 2

In this chart are represented the events which obtained with four animals. Measurements were made every ten days, beginning ten days after planting. The upper tumors are those in the control area, while the lower ones show the comparative growth obtained in the fulgurated area. Absolutely no effect of the fulguration was noted if the tumors were planted one week after the application of the spark.

If any physiological change in the nutrition of the tissues was caused by the fulguration it must have been transitory and passed coincidently with the healing of the local reaction.

active growth of spindle cells approaching the sarcomatous type. Many muscle cells and bundles are completely replaced by such cells.

Five days. The main changes are in the subdermal tissues, which are the seat of extensive purulent infiltration. There is extensive overgrowth of new spindle cells in the muscle bundles and considerable extravasated blood.

Six days. There is complete destruction of the epidermis by an abundant exudate of pus and blood. Ulceration extends to the subdermal tissues and muscles which are the seat of an active new growth of spindle cells replacing the muscle fibers and thickening the septa.

From the evidence presented as to the nature of the local reaction it seemed to us that we might expect small tumors to be decidedly influenced by fulguration, even if this were done directly through the intact skin. To test this point we chose a number of rats having recently implanted tumors about the size of a small pea and subjected them to fulguration over the tumor area with a suitable spark for a period of from 30 seconds to one minute. Tumors were carefully observed, to determine inhibition of growth or curative effects. In a few instances the tumor was sufficiently injured to prevent additional growth, and after a few days a small scab-like area, representing what had originally been the tumor, was present. Such animals may be considered as cured. In other cases there was a marked inhibition of growth but no actual cure. In these animals it must be remembered that we are dealing with very small tumors, and that only a small percentage of these could be regarded as cured by the process of fulguration. When tumors as large as a chestnut were subjected to fulguration it was found that there was a superficial area of œdema and softening, which was later followed either by ulceration or scab formation. The borders of the tumor continued to grow rapidly, and in no case was it possible to effect a cure in tumors of this size, although they were repeatedly fulgurated. From these experiments it became evident that we could not effect a cure in the rat sarcoma except in the early stages of growth, soon after implantation, and then only occasionally.

In a personal communication, de Keating-Hart stated to one of the writers that fulguration through the intact skin produced such changes as to immunize that particular area to the transplantation of experimental tumors. This point seemed to us to be of sufficient interest to warrant its confirmation. Accordingly, we fulgurated several sets of animals for periods of 30 seconds on the back, between the hips, and immediately after fulguration planted them in the fulgurated area with rat sarcoma. At the same time we planted them with the same tumor on a non-fulgurated area between the shoulders.

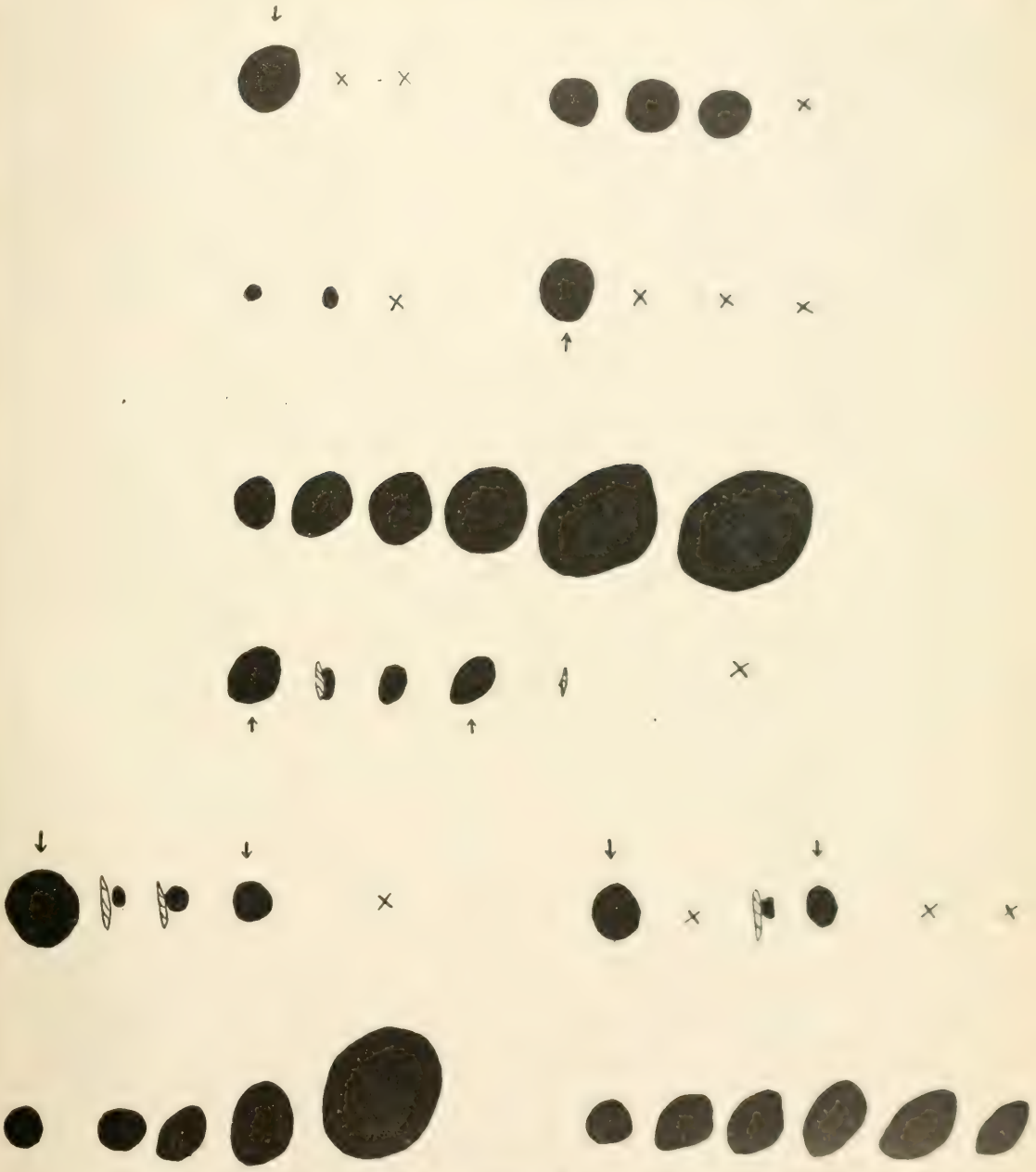

CHART 3

In this chart are shown the results obtained with five rats in which an attempt was made to cure one of the tumors by means of incomplete operation followed by fulguration. The results shown in the diagrams are typical of a large number in which similar experiments were made. The larger of the two tumors was always chosen for operation, and the operation was done upon the tumors and at the time indicated by the small arrow in the chart. The measurements were made at regular intervals. The tumors can be cured by this method, but not always at the first attempt; and thus precaution must be taken not to leave too large a fragment.

From the previous experiments quoted it seems probable that the cure is dependent upon two factors, viz.: direct injury to the tumor by the spark and the leucocytic reaction following its application.

This experiment was repeated many times, in order to rule out accidental errors; the accompanying chart is made from typical results and shows the various fates of the implanted grafts (Chart 1). In the large majority of the animals the graft planted in the fulgurated area did not grow, while the graft planted in the non-fulgurated area took in the usual way. If by any chance the graft was placed just at the border of the area of reaction following fulguration, it took as readily as the graft in the non-fulgurated area. In a very few instances the graft in the fulgurated area showed positive growth. It was, however, apparent that a tumor graft introduced directly into the area of reaction immediately following fulguration was, in a high percentage of cases, destroyed and absorbed without producing tumor growth.

In order to determine whether this effect was due to the immediate reaction or to some permanent change in the nutritive condition of the fulgurated area, a series of rats were fulgurated precisely as in the preceding experiment, but one week was allowed to elapse after fulguration before the tumor grafts were planted. At the end of this period the reaction had practically all passed, and there was only a slight infiltration of the skin to indicate the fulgurated area. The accompanying chart (Chart 2) shows that under these conditions the tumor grafts take quite as well in the fulgurated area as in the normal area. These charts give typical instances of a large number of animals treated in this way.

It must be evident from such experiments that no permanent nutritional change is produced by fulguration so as to interfere with tumor growth after the immediate local reaction has cleared up.

In the next series of experiments the tumor tissue itself was fulgurated immediately before implantation. A thin slice of freshly removed tumor about 1 mm. thick was fulgurated for 30 seconds, cut into small grafts, and planted immediately into normal rats. In about 50 per cent the grafts showed positive growth, while a non-fulgurated graft of the same tumor gave approximately 100 per cent takes. Further difference was noted in that growth of the fulgurated tumor was much slower, and there was a large percentage of regressions, indicating that the virulence was very much impaired. These results indicate that the injurious effect upon the cells directly is probably much greater than was assumed by de Keating-Hart.

Finally, a long series of experiments was undertaken to determine under what conditions the best therapeutic effect might be expected from fulguration. In these experiments grafts were planted in both the neck and hip region of the rats. When the growth was well established, one tumor was removed by operation except for a small fragment at its base. These remaining fragments were varied in size in the different experiments, in order to determine how large a fragment of growing tumor could be prevented from showing additional growth as a result of fulguration. After the operation the open wound was fulgurated for a period of 30 seconds to one minute, the spark being particularly directed to the tumor fragment left *in situ*. The accompanying chart shows typical experiments in this series (Chart 3).

In a considerable number of these experiments the fulguration of one tumor prevented growth, but it was followed by spontaneous regression of the control growth of the same animal.

The question arises here as to whether or not we have been dealing with animals in which the forces of immunity have been stimulated by the absorption of the fulgurated tumor, or whether we had just caught the tumor at a time when spontaneous regression of both tumors was about to occur. It seems hardly probable that the latter explanation could be the proper one in every instance, and there remains the definite possibility that the tumor has been injured to a sufficient degree to prevent additional growth, and its absorption has been followed by immunity. In by far the larger number of cases, however, the control tumor continued to grow, while the fulgurated tumor was either cured at the first attempt or else it recurred, as it sometimes did, as shown in

the accompanying charts, and was cured by subsequent operation and fulguration.

It became evident in this work that the fragment left to be cured by the subsequent fulguration must be a thin one, not more than 1 mm. thick, or recurrence was sure to follow. The curative effect of the spark could not penetrate deeper than that into the tumor tissue. It is evident, therefore, that although it is possible to cure rapidly growing rat sarcoma by operation and fulguration, it cannot be concluded that such happy results would follow the application of this method to growths which have infiltrated normal tissue beyond the borders of the gross tumor. It could scarcely be expected that tumor cells in the lymphatics at the border of a tumor would be seriously injured by the effect of the spark applied some millimeters distant.

It does not seem to be necessary to invoke some obscure nutritional change to explain the effect of fulguration when the severe local reaction following the spark is quite sufficient to account for the phenomena noted.

A SIMPLE AND RELIABLE METHOD OF STAINING SPORES

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The remarkable resistance of the bacterial spore to the penetration of dyes and other chemical agents has long been of interest to the bacteriologist, and many methods of staining these bodies have been devised.

Most of these methods are complicated, unreliable and especially unsatisfactory in the hands of students, whose limited time does not allow for the many repetitions usually necessary to obtain definite results.

These considerations led to the search for a simple method which would stain spores consistently in the hands of unskilled workers, and as such, be available in the class-room.

Such a method, which has been successfully employed in this laboratory for two years, is here reported.

PREPARATION OF STAIN

Four gm. of *acid* fuchsin, Grubler, are dissolved in 50 c.c. 2 per cent. watery solution of acetic acid. Two gm. of methylene blue, Grubler, are dissolved in 50 c.c. 2 per cent. watery solution of acetic acid. The two solutions are mixed, shaken and set aside for fifteen minutes. A voluminous precipitate results. The mixture is filtered through a well moistened filter-paper, and the filtrate is employed for staining. (Eosin may be used in place of the acid fuchsin, but does not give as good results.) The reddish purple filtrate will keep for several weeks, but on the appearance of a precipitate should be refiltered.

TECHNIC OF STAINING

A rather thick smear is prepared in the usual manner and fixed by heat.

The film is preferably made from an agar-culture; if broth is employed it is advisable to use a small amount of blood-serum or white of egg to fix the material to the slide.

The film is covered with a generous amount of the dye and steamed vigorously for one minute, stain being added if necessary to prevent drying.

When washed in water, the film appears stained a bright red.

The slide is now dipped a few times in a dilute solution of sodium carbonate (7 or 8 drops of a saturated aqueous solution in a tumblerful of water), and the moment the film turns

blue the action of the alkali must be interrupted by rinsing the preparation in water. The film is now ready to be dried, mounted and examined. The whole procedure takes less than two minutes.

On examination the spores will be found to stain a deep red, and the bodies of the bacteria bright blue, the intensity of the blue stain depending on the length of exposure to the alkali; too long an exposure may cause the spores to stain blue. Old spores, and particularly free spores, stain well, young spores faintly or not at all.

That the exposure to the acetic acid is the essential portion of the method is shown by steaming a film with a 2 per cent. watery solution of acetic acid and resteaming with a watery solution of any of the usual bacterial dyes. On examination the spores will be found to have taken up the stain, even if as weak a dye as Bismarck brown is employed.

By decolorization with alcohol for a few seconds to remove the stain from the bacterial bodies and then counter-staining with a contrast dye, any combination desired may be produced.

The general application of this method seems to be established by the successful results obtained on the following spore-bearing strains: *Bacillus subtilis, B. anthracis, B. megaterium, B. sporagenes, B. lacti morbis, B. putrificus, B. oedematis maligni, B. tetani, B. mesentericus, B. cereus,* and the bacillus of *symptomatic anthrax.* A failure must be recorded with the *B. mycoides,* but this strain also proved resistant to all the standard methods.

The advantages claimed for this method are its simplicity and reliability.

It may be of interest to those engaged in teaching to know that this method has been used in class-room work for the past two years and has yielded uniformly reliable results.

A number of the strains employed were obtained from the collection at the American Museum of Natural History, for which I desire to express my thanks to Prof. C. E. A. Winslow.

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